Spatio-temporal variations in leaf herbivory within a canopy of *Fagus crenata* and their causal factors

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CONTENTS

Chapter 1: Spatial variations in leaf herbivory within a single tree canopy and their possible causes

Trees are highly variable in their characteristics (Denno and McClure 1983). Even within a single tree canopy, spatial variations are observed in characteristics of leaves. Microenvironments of leaves such as light intensity and temperature are heterogeneous within a canopy, and this heterogeneity produces within-tree, spatial variations in leaf characteristics.

Within-tree variations in leaf characteristics are closely related to the pattern of performance of insect herbivores and this correlation causes spatial variations in leaf herbivory. The effect of the lower trophic level's organisms, i.e. plants, on herbivores is called a bottom-up effect. Although organisms of the higher trophic level, i.e. natural enemies, have top-down effects on the performance of herbivores, this study focused on the bottom-up effect and within-tree variations in herbivory level caused by such an effect.

Causes of bottom-up effects on within-tree variations in leaf herbivory level can be classified broadly into the following three categories: plant environmental properties, plant morphological characters and plant internal characters. Environmental properties that are considered to cause within-tree variations in herbivory level include height, direction, temperature and light intensity. They can be rephrased as abiotic factors. Branches, shoots and leaf orders are listed as among plant morphological characters which have effects on variations in herbivory. They are also called visible biotic factors. Plant internal characters

include leaf mass per area, water, nitrogen and phenolic concentrations of leaves. They are also known as invisible biotic factors. These factors in each category have direct and/or indirect effects on within-tree variations in herbivory level (Fig. 1-1).

Plant environmental properties themselves involve spatial factors. In other words, plant environmental properties vary spatially, and their effects on herbivores on trees cause within-tree variations in herbivory level. In Fig. 1-1, these direct effects of plant environmental properties are expressed as type A pathway. Similarly, since plant morphological characters vary spatially within a tree canopy, direct effects of plant morphological characters on herbivores also cause herbivory level's spatial variations (type B pathway in Fig. 1-1).

There are three kinds of indirect effects on herbivores that cause spatial variations in herbivory level (types C, D, E pathways, in Fig. 1-1). Some kinds of plant morphological characters are determined by their environmental properties which vary spatially. Accordingly, plant morphological characters also show spatial variations and so effects of morphological characters on herbivores produce spatial variations in herbivory level (type C pathway, Fig. 1-1).

Leaf characteristics such as toughness and thickness change with time, and generally old leaves are tougher and thicker than new leaves. In the case of evergreen tree species whose leaf age is more than one year, leaves of different ages, i.e. leaves with different characteristics, are scattered within a canopy. When such spatial variations in leaf internal characters, which are caused by spatial variations in morphological characters, have effects on herbivores, within-tree, spatial variations are observed in herbivory level (type D pathway, Fig. 1-1).

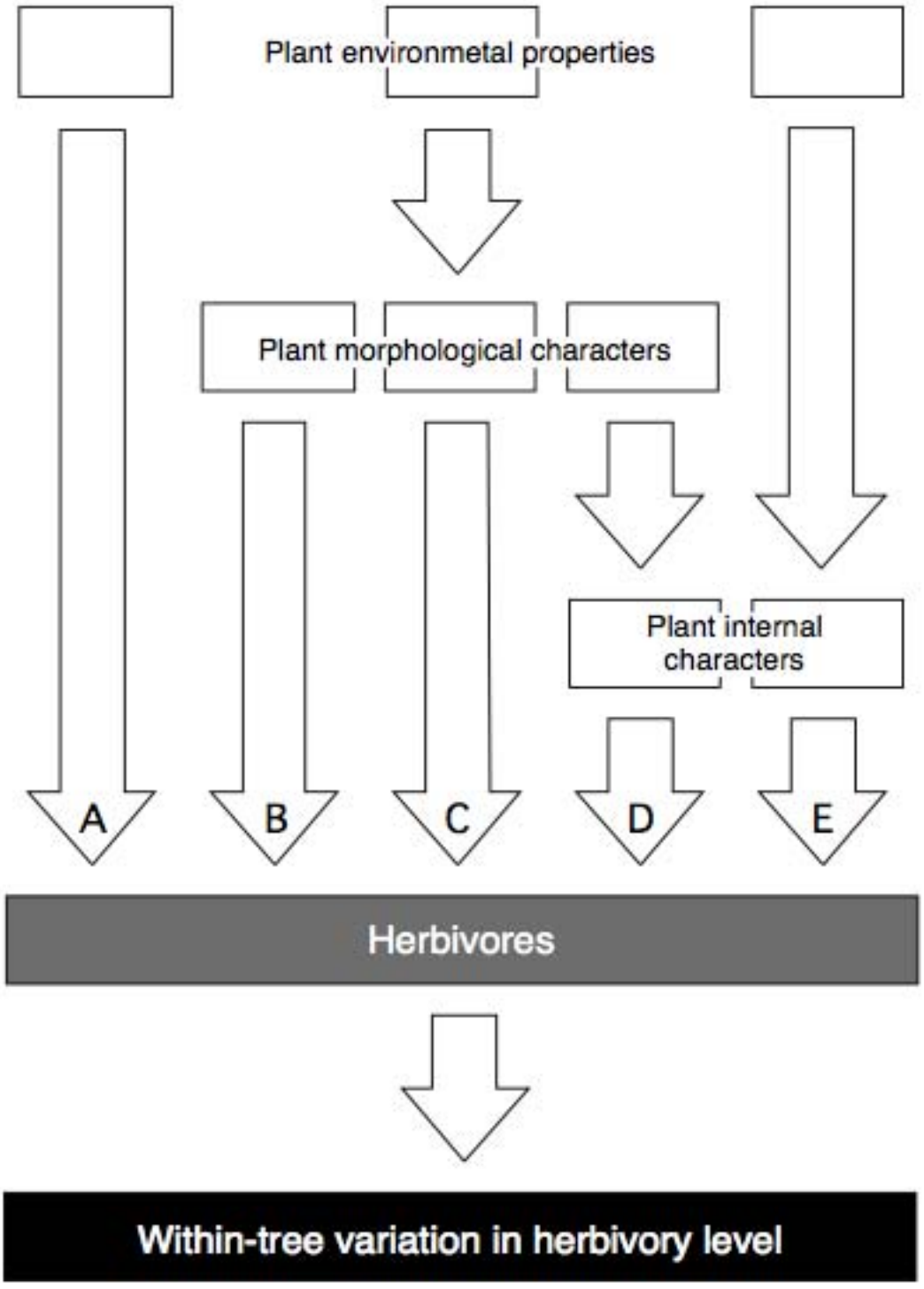

Fig. 1-1 Categories of the way of effects of plant characteristics on herbivores. Arrows with alphabetical letters show direct or indirect effects of plant environmental properties, plant morphological characters and plant internal characters on herbivores.

As is seen in the difference in characteristics between sun leaves and shade leaves, plant internal characters change spatially in accordance with spatial variations in plant environmental properties such as light intensity. Herbivores are also affected by these spatial variations in plant internal characters, and as shown by type E pathway in Fig. 1-1, this effect produces within-tree variations in herbivroy level.

I will now review previous studies on within-tree variations in herbivore's performance and their causal factors. According to the classification of causal factors described above and in Fig. 1-1, the following review is composed of five sections (from ***A*** to ***E***).

A. Direct effects of plant environmental properties

Compared with herbaceous plants, trees are tall and resources for herbivores are distributed widely in vertical extent. For small insects whose flying capacity is low, leaves in higher positions are unavailable. This kind of direct effect of height on herbivores is observed in gall makers. Several studies reported high gall densities in lower positions of canopies (Askew 1962; Rosenthal and Koehler 1971). (However, gall distribution is not always explained by direct effects of height (Kampichler and Teschner 2002).)

In the case of Japanese beetles, *Popillia japonica* Newman, their aggregation and heavy feeding were observed in upper canopies of their host plants. Since these phenomena were not explained by host nutritional variation, direct effects of height (Rowe and Potter 1996) or temperature (Kreuger and Potter 2001) on beetles were suggested.

Leaves of trees are also distributed widely in horizontal extent. Studies on western tent caterpillars, *Malacosoma californicum pluviale*

Dyar, showed their preference for the sunny side of host trees as their oviposition site, and environmental factors such as light and temperature were suggested as important factors that determine their oviposition preference (Moore *et al.* 1988). Similarly, silk tents of the tent caterpillars, *Yponomeuta mahalebella* Latr, were preferentially made in a southward orientation, and this preference was related to thermal differences caused by light conditions between the inside and the outside of the tents (Alonso 1997).

In the case of tall trees, wind speed increases considerably in upper positions of canopies. Studies on the felted beech scale, *Cryptococcus fagisuga*, revealed that a decrease in abundance of these scale insects larvae with increasing height was closely related to variation in wind speed within the canopy (Wainhouse 1980).

B. Direct effects of plant morphological characters

A tree is composed of branches, a branch is composed of shoots and several leaves are attached to one shoot. There are variations in leaf characteristics both among different branches and shoots. Leaves themselves vary in their characteristics according to their ages. In the case of evergreen tree species whose leaf age is over one year, leaves of different ages are mixed within a canopy. In the case of deciduous tree species whose leaf age is within one year, variations in leaf age, although on a smaller scale, are also observed, especially in trees whose leaf emergence pattern is successive type. Thus, variations at different levels within a tree have effects on herbivores.

Suomela *et al*. investigated the within-tree variability in leaf

characteristics of the mountain birch and their effects on herbivores (Suomela and Ayres 1994; Suomela and Nilson 1994; Suomela *et al.* 1995a; Suomela *et al.* 1995b; Suomela 1996). They sampled leaves, with trees, ramets within trees, branches within ramets, short shoots within branches, and leaves within short shoots as the sampling levels. Their results showed significant effects of plant morphological characters, such as ramets within the trees and branches within the ramets, on variations in leaf characteristics and herbivores.

Variations in shoot length also affect hervibores. The phenomenon in which the bud-galling sawfly, *Euura mucronata*, attacks longer shoot length classes on its host, *Salix cinerea*, more frequently than shorter shoots is explained by the successful establishment of larvae in galls on longer shoots (Price *et al.* 1987).

Most eucalypt species are heterophyllous, with their foliage undergoing distinct morphological and chemical changes between adult and juvenile growth, and this hererophyllous characteristic affects herbivores. In the case of paropsine chrysomelid beetles, *Chrysophtharta agricola*, their oviposition preference for juvenile foliage over adult foliage was observed (Nahrung and Allen 2003). Similarly, the autumn gum moth, *Mnesampela privata*, oviposited its eggs preferentially on juvenile leaves of its *Eucalyptus* host trees.

C. Indirect effects of plant environmental properties through changes in plant morphological characters

A tree canopy is composed of many shoots, and different types of shoots are mixed within canopies of some tree species. For example,

proportions of long shoots may be higher at the top of canopies than those at the bottom of canopies. As a result, herbivores which prefer long shoots as their oviposition site may be indirectly affected by heights of trees.

In the case of *Fagus crenata*, although there is no clear difference in shoot type, there were variations in shoot length and number of leaves within a canopy (see Chapter 4). Proportions of longer shoots with many leaves increase with increasing height, and this may have some effects on folivores. Considering that longer shoots are bigger in size at their bud stage, herbivores which oviposit eggs on buds may also be affected indirectly by heights of trees.

D. Indirect effects of plant morphorogical characters through changes in plant internal characters

When leaves of different ages are mixed in a canopy, preferential feeding of new leaves by herbivores is often observed. This kind of preference was confirmed by laboratory experiments of rearing herbivores with new and old leaves. Low survival and slow development on mature leaves of *Eucalyptus blakelyi* Maiden, which were more than three times as tough as new leaves, were reported on the leaf beetle, *Paropsis atomaria* Oliver (Larsson and Ohmart 1988). In laboratory feeding trials with six leaf beetle species, young leaves of *Populus tremula* L., *Salix phylicifolia* L. and *S. pentandra* L. were invariably preferred (Ikonen 2002). The oviposition preference of adult females of *Chrysophtharta bimaculata* for new leaves over old ones was explained by differences in leaf toughness (Howlett *et al.* 2001).

On the contrary, the preference for old needles over new ones was observed on the last instar larvae of pine moth, *Dendrolimus spectabilis* Butler, and rearing experiments confirmed this trend (Togashi and Takahashi 1977b, a). Plant internal characters involved in the interaction between pine moths and their host tree, *Pinus thunbergii* Parl, were not investigated.

E. Indirect effects of plant environmental properties through changes in plant internal characters

According to studies on the correlation between light intensity and phenolic contents of leaves with four west African rain-forest plant species (Mole *et al.* 1988; Mole and Waterman 1988), shaded foliage with low phenolic contents was in general nutritionally more acceptable food for herbivores. In this case, light intensity affects herbivores indirectly through changes in phenolic contents of leaves.

Experimental shading of branches of mountain birch trees caused changes in leaf characteristics such as water content, toughness, sugar content, total phenlics content and soluble proanthocyanidins content (Henriksson *et al.* 2003). *Epirrita autumnata* larvae grew better in leaves from experimentally shaded branches, and this indicates effects of experimental shading, i.e. changes in light intensity, on herbivores through changes in leaf characteristics.

Rearing experiments of *Malacosoma disstria* Hbn larvae with leaves from different parts of a canopy showed high larval performance when they fed on leaves collected in the upper crown, and this was explained by high total nitrogen content in the upper crown's leaves (Fortin

and Mauffette 2002). This suggests that light affects herbivores positively through changes in leaf nitrogen content. Similarly, positive relations between light and herbivore performance were observed on butterfly larvae, *Euphydryas chalcedona*. The amount of herbivory by *E. chalcedona* larvae on *Diplacus auranticus* shrubs was related to the light intensity that the shrubs received and to leaf characteristics that vary with light intensity, e.g. leaf specific weight (Lincoln and Mooney 1984).

Light is not the only factor that causes within-tree variations in leaf characteristics and herbivory level. There are differences in leaf quality among branches in different positions. Partial defoliations of a tree canopy by leafcutting ants, *Atta columbica*, were explained by within-tree, patchy variations in leaf quality such as leaf moisture and phenolic content (Howard 1990).

As reviewed above, within-tree, spatial variations in herbivory level are observed in canopies of various tree species, and various abiotic and biotic factors are recognized as causes of these variations. These factors change temporally as well as spatially. For example, leaves become tough as time passes after leaf flush. In the case of trees with long leaf life span, older leaves are very different from younger ones in their toughness, water and chemical contents and so on. This suggests that the patterns of spatial variations in plant characteristics described above also change temporally. Accordingly, it can be supposed that spatial variations in herbivory level also change temporally. In this research, I investigated little-studied temporal changes in spatial variations in leaf herbivory within a canopy of *Fagus crenata*. Making the best use of a tree tower constructed around the observed canopy, I conducted

studies on insect-plant interactions from the plant side.

The following is an outline of the chapters. In Chapter 2, I conducted continuous, non-destructive observations of leaves in various positions within the canopy of *F. crenata*, and recorded changes in leaf area consumed by herbivores. Effects of light intensity on leaf herbivory level through changes in leaf characteristics were discussed with the obtained results. In Chapter 3, I introduced another causal factor, that is, leaf order, of within-tree variations in herbivory level, and considered seasonal changes in causal factors of within-canopy variations in leaf area consumed by herbivores. In Chapter 4, I focused on midge galls produced on the leaf surface of *F. crenata* immediately after leaf flush, and examined their within-tree distributions. Relationships between gall distributions and within-canopy variations in leaf phenology of *F. crenata* were discussed. In the final chapter, I summarized the results of the previous chapters, and explored seasonal changes in patterns of within-tree variations in herbivory level and their possible causes.

Chapter 2: Heterogeneous light environments within a canopy of *Fagus crenata* and their indirect effects on herbivores

2.1 Introduction

Over the course of their lifetime tree leaves change their traits, such as altering their toughness or chemical composition. Temporal variation in leaf characteristics produces complex interactions between plants and herbivores. Young leaves, especially immediately after their emergence, are soft, and are vulnerable to insect herbivores. However, they become harder with time, and accordingly their palatability for herbivores decreases (Feeny 1970). Because of temporal changes in leaf characteristics, the suitability as a food for insect herbivores differs among leaves of different ages (Ayres and Maclean 1987; Damman 1987; Thomas 1987; Larsson and Ohmart 1988). Several studies thus suggest that herbivores cannot develop successfully outside a well-defined phenological window of opportunity for using valuable leaves (Hunter and Lechowicz 1992; Lawrence *et al.* 1997).

Spatial variations are also observed in leaf traits within the canopy of a single tree. On a tall tree composing part of a forest canopy, microenvironments for leaves are remarkably different among shoots, and so leaves in different canopy layers vary in their characteristics. Such spatial change in leaf traits leads to preferential feeding by herbivores (Zucker 1982; Howard 1990; Wallin and Raffa 1998). By rearing insects on leaves sampled from the lower and upper regions of trees, Fortin and Mauffette (2002) found significantly higher insect performance, i.e. larger

pupal masses and a greater number of eggs produced, among larvae fed with leaves from the upper part of the crown.

Among extrinsic factors that change spatially (Orians and Jones 2001), light availability is the most noticeably different within an individual tree. Because light is one of the major factors that regulate photosynthesis, variation in light causes variation in leaf characteristics such as the carbon concentration and the carbon/nitrogen ratio. According to the CN balance hypothesis (Bryant *et al.* 1983; Coley *et al.* 1985), changes in the carbon/nutrient (such as nitrogen) ratio correlate with levels of plant defensive chemicals, such as phenolic metabolites. Some studies verified this hypothesis by documenting the indirect relationship between light and herbivores through the intermediary of phenolic plant chemicals (Larsson *et al.* 1986; Lindroth *et al.* 1993; Dudt and Shure 1994; Agrell *et al.* 2000; Nabeshima *et al.* 2001).

The studies cited above investigated either temporal variations or spatial variations. However, this kind of research does not make it clear whether and how patterns of spatial variations in leaf characteristics change temporally. Such spatial differences are expected to change with time. For example, a difference in leaf characteristics found between different layers of a canopy at one time is not necessarily observed at another time of the year. We need to examine the factors causing such a temporal change in the pattern of spatial variation. It is difficult, however, to describe them accurately by sampling leaves destructively for each observation. A non-destructive sampling method, which makes it possible to observe the same leaves on the same trees throughout the year, is necessary for investigating how spatial variations in leaf traits are affected by time.

The objective of this study, therefore, was to investigate how leaves

under different light conditions within a tree change their characteristics, and how levels of leaf herbivory vary throughout a year. This was the first study to clarify temporal and spatial variations in leaf herbivory level within the canopy of a single tree by adopting a non-destructive sampling method.

2.2 Materials and Methods

This research was conducted at the Kyoto University Forest in Ashiu, located in the northeastern part of Kyoto Prefecture, Japan (35°18'N, 135°43'E). The mean annual temperature in 2001 was 12.3 °C, and the mean monthly temperature ranged from –0.7 °C in January to 25.5 °C in August. The annual precipitation in 2001 was 2548 mm, and the monthly rainfall ranged from 56 mm in April to 380 mm in August. The forest mainly consisted of natural mixed stands of *Cryptomeria japonica* D. Don var. *radicans* Nakai, *Fagus crenata* Blume, *Quercus crispula* Blume and other deciduous species. These were the dominant species which constituted the forest canopy.

The observed tree was a *F. crenata*. The tree measured 17.2 m in height by 60 cm in diameter at breast height. Leaves were distributed from 3 m above ground to the top of the tree, but they were mainly concentrated in the top half of the canopy. A tower, constructed of steel pipes and steps, was built around the tree crown so that we could observe leaves at various heights without tearing them off.

A leaf cluster was defined as a group of leaves attached to a branch about one meter long. Twenty-four leaf clusters were selected from various layers of the tree crown so as to cover the wide range of photon flux densities. Five twigs were chosen from each branch for observation. Each twig had from three to 49 current-year shoots and from 11 to 152 leaves.

First, I identified all 6,040 leaves attached to the five twigs in each of the 24 clusters by drawing a sketch of them. Then, observations of the leaves were carried out once a week from early May to late November in

2001, that is, from after the leaves flushed until they fell. At every observation, I checked the leaves for a mark of leaf herbivory. I used a digital camera to take pictures of the leaves which had been eaten by herbivores. Then, the area of each leaf was measured, and its original area, before consumption by herbivores, was estimated with the public domain NIH Image program (developed at the U.S. National Institutes of Health). These results were used to calculate the ratio of the eaten leaf area to the original leaf area for each leaf. Assuming that all leaves on each twig, whether eaten or not, originally had the same area, I computed the ratio of the eaten leaf area to the total area for each twig.

On an overcast day in early September, 2001, I simultaneously measured the photosynthetic photon flux density at the top of each leaf cluster and that at the top of the canopy using light meters (LI-COR, LI-190SA). The relative photosynthetic photon flux density (RPPFD) of each leaf cluster was then calculated.

Ten leaves were collected from each leaf cluster on June 13, August 16, and October 16, 2001, and on April 17, April 30, and May 22, 2002. Leaves of the upper layer had fallen off by October; hence the samples collected in October were limited to leaves from ten leaf clusters. The leaf area of each sample was measured with a digital camera and the photo retouch software mentioned above. The samples were dried for two weeks at 40°C, and the leaf mass per area (LMA) of each sample was calculated. Dried samples from 2001 were ground with a mill, and their carbon, nitrogen, total phenolics and condensed tannin concentrations were measured. The carbon and nitrogen concentrations were measured with a CN corder (Yanaco, MT-600). The sample leaf powders were extracted with 50% methanol for 24 hours, and their total phenolics concentrations

were quantified with a spectrophotometer (Shimadsu, UV-1200), using tannic acid as a standard (Price and Butler 1977; Waterman and Mole 1994). Their condensed tannin concentrations were also quantified with a spectrophotometer (Porter *et al.* 1986), using cyanidin chloride as a standard.

Leaves from other individual trees were also collected to conduct replicate data sampling of the primary tree. Two additional *F. crenata* trees, each 18 m high, were selected near the primary tree. I collected three branches about 80 cm long from each of the canopy regions: top, middle, and bottom, in mid-June and mid-August, 2001. I chose four twigs with about 30 leaves from each branch, and we measured the area consumed by herbivores, the dry mass, and the total area of the leaves. Then, the values of consumed leaf area and those of LMA in the three different canopy layers were compared with each other using ANOVA and Tukey's multiple comparison test. Statistical analyses were made by SAS (SAS 1985).

2.3 Results

CLA and LMA of leaves of the tower-surrounded tree

Leaves of *Fagus crenata* flushed at the end of April, and all of them fell in late November, 2001. In 2002, leaves flushed in mid-April, about two weeks earlier than in 2001. Fig. 2-1 shows the seasonal changes in the percentage of consumed leaf area (CLA) of each leaf cluster within the *F. crenata* canopy in 2001. CLA started increasing in May, i.e. immediately after leaf flush, in all clusters, each varying in the rate of increase (Fig. 2-1). CLA at the end of May ranged from 0.4 to 3.3%. In bright clusters in the upper layers of the canopy, where values of RPPFD were more than 15%, no further consumption was observed after June (Fig. 2-1). On the other hand, CLA continued to increase after June in most clusters in the lower part of the canopy, where RPPFD was less than 15% (Fig. 2-1).

Fig. 2-2 indicates the relationships between CLA and RPPFD of all clusters. Although no clear relation between light and CLA was observed in May, a significant exponential decrease in CLA was observed after June, coordinated with the increasing RPPFD (R^2=0.53, $p<0.01$) (Fig. 2-2).

LMA of each leaf cluster is shown in relation to its RPPFD in Fig. 2-3. LMA increased with an increase in RPPFD under low light conditions, and reached rather constant values at high RPPFD in June, August, and October, 2001 (Fig. 2-3a). There was no variation in LMA among leaf-sampling times in 2001, i.e. in June, August, and October (Fig. 2-3a). A marked increase in LMA with light was not observed in April, 2002 (Fig. 2-3b). The LMA values in April were smaller than those in May, 2002 and than

those in June, August, and October 2001 (Fig. 2-3a,b). In May 2002, a gradient of LMA values against the RPPFD of the clusters was observed (Fig. 2-3b).

CLA and LMA of leaves of other individual trees

Table 2-1 shows CLA of leaves at the top, middle, and bottom of the two tree canopies, sampled in June and August as replicates for the primary tree. On Tree A, CLA was not different among the three canopy layers in June (Table 2-1). In August, however, CLA in the bottom layer was significantly larger than that in the upper two layers (Table 2-1). Moreover, CLA increased from June to August in the bottom layer, but CLAs did not do so in the top and middle layers (Table 2-1). A similar tendency was also exhibited on Tree B, though a significant difference was found in June between the middle and bottom layers.

Table 2-2 shows LMA of leaves collected from the top, middle, and bottom of the canopies of Tree A and Tree B in June and August. Significant differences in LMA of leaves were observed among the three different canopy layers on Tree A as well as Tree B both in June and August (Table 2-2).

Chemical analysis of leaves

Fig. 2-4 illustrates the relationships between RPPFD and the three leaf traits: carbon concentration, nitrogen concentration, and C/N ratio. Although the carbon concentration of leaves was about 50% in all leaf clusters, it increased slightly as RPPFD increased (r=0.79 in June, r=0.76 in

August, $p<0.01$, Fig. 2-4a). The nitrogen concentration of these leaves ranged from 1.3% to 2.2%, and decreased as light intensity increased ($r=-0.78$ in June, $r=-0.83$ in August, $p<0.01$, Fig. 2-4b). Consequently, the C/N ratio also increased with the RPPFD ($r=0.81$ in June, $r=0.81$ in August, $p<0.01$, Fig. 2-4c).

Fig. 2-5 illustrates the relationship between the concentration of leaf secondary metabolites and the RPPFD. Although there was large variation in the concentrations observed, the total phenolics concentration was higher in leaves under high RPPFD than that of leaves under low RPPFD (Fig. 2-5a). The relationship between the condensed tannin concentration and the RPPFD was more apparent. The condensed tannin concentration of the leaves increased with an increase in RPPFD, and the value at the top of the canopy was about three times as large as that at the bottom (Fig. 2-5b).

Finally, Table 2-3 summarizes the correlation coefficients of the CLA, RPPFD, and measured leaf characteristics. There were strong positive or negative correlations between the CLA and the measured leaf traits, except for the total phenolics concentration (Table 2-3).

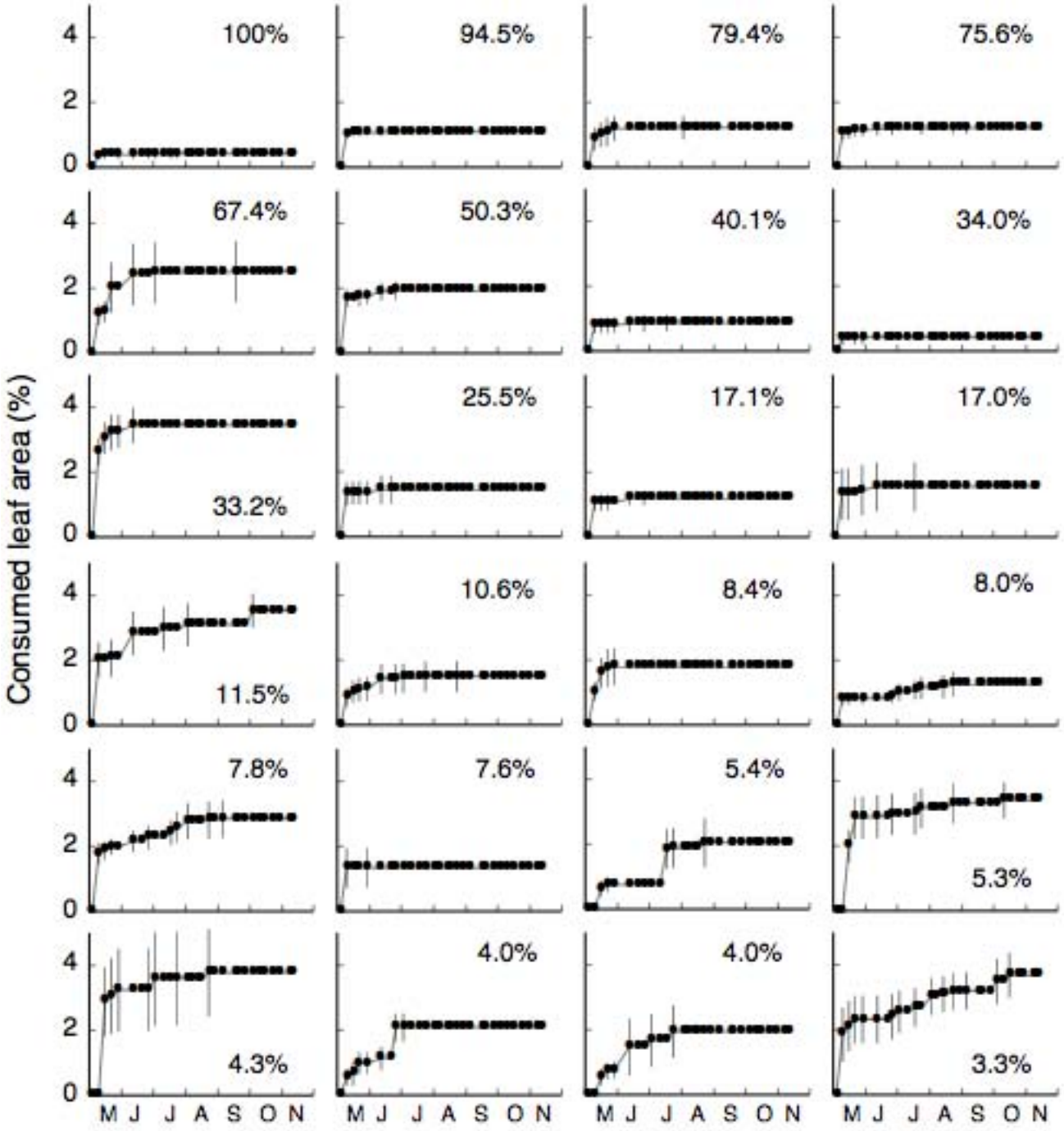

Fig. 2-1 Seasonal changes in the percent of the leaf area consumed in 24 leaf clusters within the canopy of the tower-surrounded *Fagus crenata* in 2001. Data shown are the mean values of five twigs. Numerals in each panel show the relative photosynthetic photon flux density of the top of each leaf cluster as compared to the top of the canopy. Vertical bars show the standard errors among the five twigs, but when the mean value and the standard error are the same as those of the preceding observation date, only the mean value is shown.

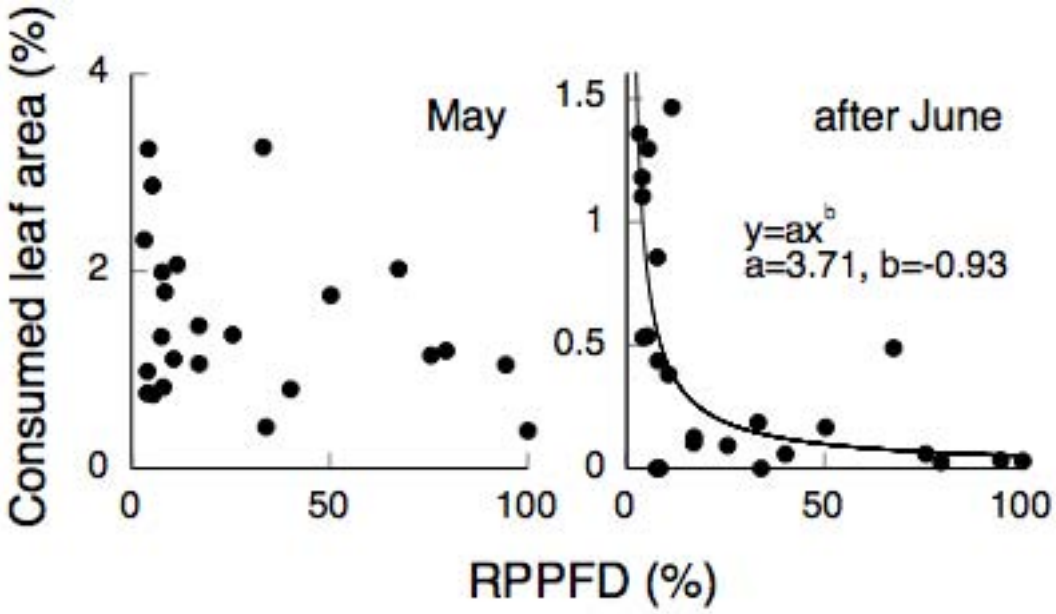

Fig. 2-2 The relationship between the percentage of the leaf area consumed and the RPPFD in May (left), and after June (right). The mean value of each cluster was shown.

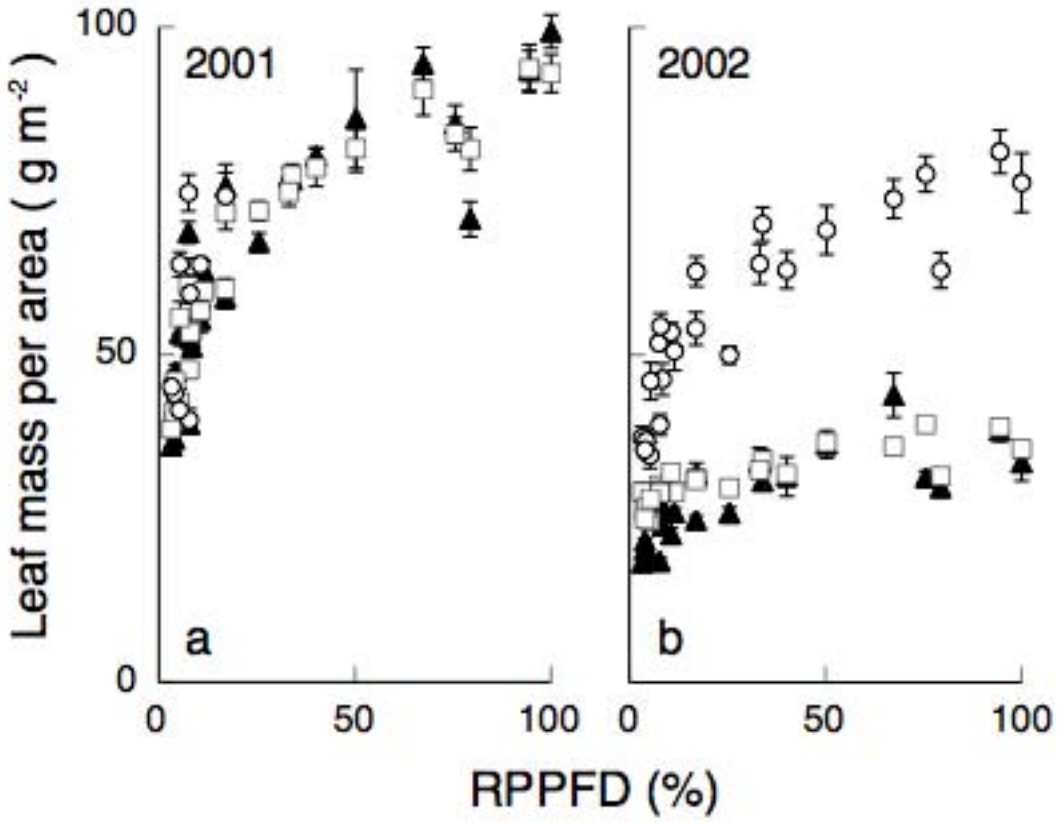

Fig. 2-3 The relationship between the mean value of leaf mass per area and the RPPFD of each leaf cluster in June (solid triangle), August (open square), and October (open circle) in 2001 (left) and in mid-April (solid triangle), late-April (open square), and mid-May (open circle) in 2002 (right). Vertical bars show standard errors (n =10).

Table 2-1 Percent of leaf area consumed by herbivores in the top, middle, and bottom of the canopy of two replicate trees. Data are shown in mean ± standard error of each twig (n=12).

	Tree A		Tree B	
	June	August	June	August
Top	0.8 ± 0.2 **a**	1.9 ± 0.4 **a**	2.4 ± 0.6 **ab**	2.3 ± 0.6 **a**
Middle	1.2 ± 0.3 **a**	3.0 ± 0.6 **a**	1.6 ± 0.3 **a**	2.4 ± 0.8 **a**
Bottom	1.8 ± 0.6 **a**	4.9 ± 0.6 **b**	3.8 ± 0.5 **b**	5.9 ± 0.6 **b**

Means with the same letter are not significantly different (ANOVA and Tukey's multiple comparison test, P = 0.01).

Table 2-2 LMA of leaves collected from different canopy layers of two replicate trees. Data are shown in mean ± standard error of each twig (n=12).

	Tree A		Tree B	
	June	August	June	August
Top	69.5 ± 1.3 **a**	69.9 ± 1.8 **a**	89.3 ± 5.3 **a**	96.8 ± 3.6 **a**
Middle	42.7 ± 0.6 **b**	45.8 ± 0.6 **b**	63.6 ± 1.9 **b**	53.3 ± 1.8 **b**
Bottom	39.2 ± 0.8 **c**	39.0 ± 0.5 **c**	48.3 ± 1.1 **c**	43.8 ± 1.0 **c**

Means with different letters are significantly different (ANOVA and Tukey's multiple comparison test, P = 0.01).

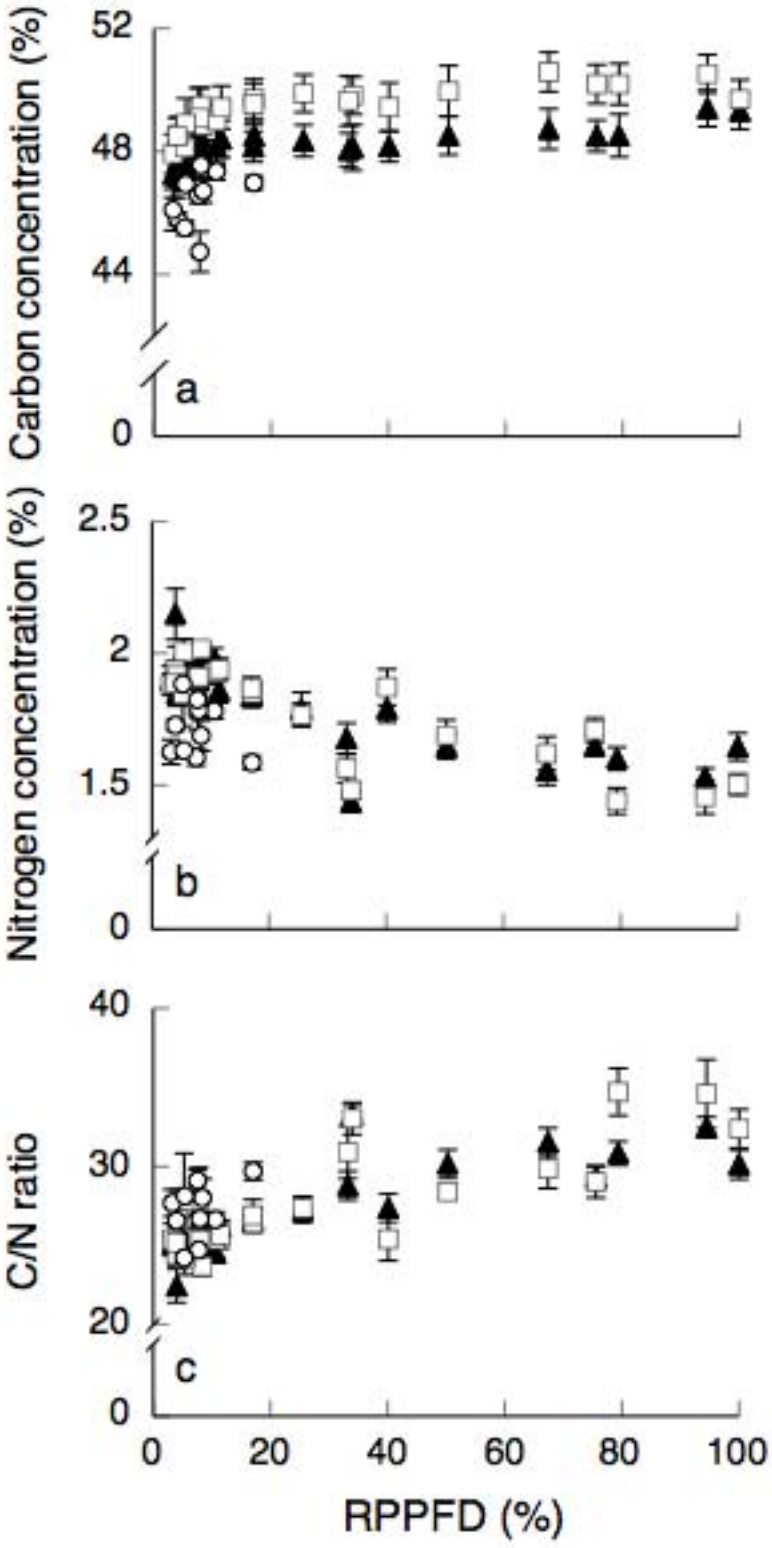

Fig. 2-4 The relationship between RPPFD and the mean value of the carbon concentration (a), nitrogen concentration (b), and C/N ratio (c) in each leaf cluster in June (solid triangle), August (open square), and October (open circle). Vertical bars show standard errors (n =10).

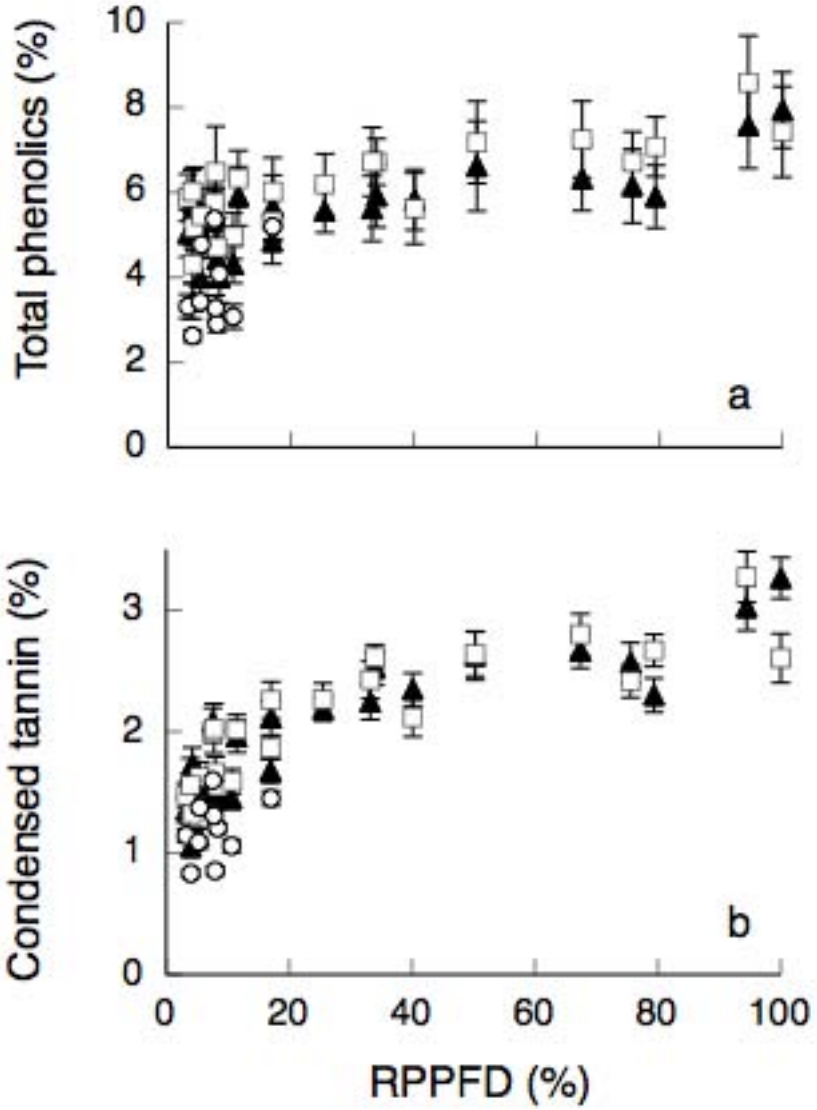

Fig. 2-5 The relationship between RPPFD and the mean value of the total phenolics concentration (a) and condensed tannin concentration (b) in each cluster in June (solid triangle), August (open square), and October (open circle). Values of the total phenolics concentrations are equivalent to tannic acid. Values of the condensed tannin concentrations are equivalent to cyanidin chloride. Vertical bars show standard errors (n =10).

Table 2-3 Correlations between consumed leaf area after June, RPPFD, and measured leaf characteristics of the tower-surrounded tree in June. Arcsin transformed values were used for consumed leaf area and RPPFD.

	Consumed leaf area (%)	RPPFD (%)	LMA ($g\ m^{-2}$)	C (%)	N (%)	C/N ratio	Total phenolics (%)	Condensed tannin (%)
Consumed leaf area (%)	1.00							
RPPFD (%)	- 0.54 **	1.00						
LMA ($g\ m^{-2}$)	- 0.61 **	0.86 **	1.00					
C (%)	- 0.54 **	0.79 **	0.83 **	1.00				
N (%)	0.54 **	- 0.78 **	- 0.81 **	- 0.67 **	1.00			
C/N ratio	- 0.56 **	0.81 **	0.84 **	0.70 **	- 0.99 **	1.00		
Total phenolics (%)	- 0.33	0.80 **	0.80 **	0.79 **	- 0.79 **	0.79 **	1.00	
Condensed tannin (%)	- 0.58 **	0.90 **	0.96 **	0.85 **	- 0.87 **	0.89 **	0.92 **	1.00

** = $P < 0.01$

2.4 Discussion

In this study, repeated observations of *Fagus crenata* leaves revealed how patterns of spatial variations in leaf herbivory change with time. Herbivores started eating *F. crenata* leaves immediately after leaf flush in all leaf clusters within the canopy. Further increases in consumed leaf area (CLA) observed after June, 2001 were found mainly at the bottom of the canopy, where light availability was lower (Fig. 2-1, 2-2). Replicate data obtained by destructive samplings showed a similar tendency. Although CLAs before June at the bottom of the canopy were not significantly different from those at the top, CLAs before August from the bottom were significantly higher than those at the top (Table 2-1). It follows from this that there was significantly higher consumption of leaves at the bottom of the canopy after June.

Within-tree, temporal variations in CLA found in this research can be partially explained by variations in LMA, probably caused by the heterogeneity of the light. Although LMA data immediately after bud-burst were not obtained in the same year (2001) but in the next year (2002), they may be used to complete the year-round change in leaf traits. In April, as is shown in Fig. 2-3b, leaf mass per area (LMA) was rather low in all leaf clusters within the canopy. It is probable that *F. crenata* leaves immediately after leaf flush are easy for insect herbivores to ingest irrespective of their position in the canopy. However, the values of LMA increased in all clusters after late-April, and LMA obviously increased with an increase in RPPFD after May (Fig. 2-3a, 2-3b). Table 2-3 shows that there was a strong positive correlation between the values of LMA in June and RPPFD. Furthermore, there was a strong negative correlation

between CLA and LMA (Table 2-3). Similar tendencies were also recognized in the two individual trees. At the bottom of the trees' canopies, where the LMA of the leaves was significantly lower than that of leaves in higher positions (Table 2-2), the CLA was significantly higher than that in higher canopy layers in August (Table 2-1). These observations give support to the general recognition that leaves are defended against herbivores by toughness (Coley 1983; Reich *et al.* 1991; Choong 1996). Considering the discussions in this and the preceding paragraph together, it is likely that readily available food for herbivores is different among leaf clusters under different light conditions at different times, and this explains the occurrence of variation in CLA with time and within an individual tree (Fig. 2-2, Table 2-1).

There was a strong positive correlation between the RPPFD and C/N ratio (Table 2-3). Furthermore, positive correlations were also found between the C/N ratio and secondary metabolites of leaves, that is, total phenolics and condensed tannin (Table 2-3). Several studies revealed similar effects of sunlight on defensive chemicals of leaves (Larsson *et al.* 1986; Lindroth *et al.* 1993; Dudt and Shure 1994; Agrell *et al.* 2000). The relationship between light and leaf secondary metabolites found in this study, i.e. the increase in leaf secondary metabolites with the RPPFD through an increase in the C/N ratio, supports the CN balance hypothesis that under high light and limiting nutrient levels, carbon becomes relatively more available for defense investment (Bryant *et al.* 1983; Coley *et al.* 1985). This hypothesis was originally proposed for different plants under various light and nutrient conditions. It is clarified in the present study that this hypothesis is also applicable to the variation in leaf traits among different leaf clusters within a single canopy. At the top of a canopy,

where leaves are exposed to plentiful sunlight, carbon is more available for carbon-based secondary metabolites, such as tannin, than at lower layers of the canopy.

A significant negative correlation was found between RPPFD and nitrogen concentration, while there was a significant positive correlation between nitrogen concentration and CLA (Table 2-3). It follows from these results that light indirectly influences food availability for herbivores by varying the quantity of nitrogen. The positive correlation between nitrogen and CLA lends support to Mattson's (1980) argument that the lower concentration of nitrogen in leaves means low food value for herbivores. Thus the observed within-tree decrease in leaf nitrogen concentration partly accounts for reduced consumption in leaves at the top of the canopy (Fig. 2-1).

Although there was no significant relationship found between the total phenolics concentration and CLA, the condensed tannin of leaves had significant negative effects on CLA (Table 2-3). Tannin is considered to work as an inhibitor of digestion for herbivores (Rhoades and Cates 1976; Schultz 1989), and has a negative influence on herbivores, causing slow growth and high mortality (Feeny 1968; Ayres *et al.* 1997; Nomura and Itioka 2002). For example, Ayres *et al.* (1997) reported that the growth rate of leaf beetles was 30% lower when fed on leaves painted with condensed tannin solution, which is equivalent to 3% tannin in dry mass, than when fed on leaves without it. At the top of the canopy of *F. crenata*, the level of condensed tannin in leaves after June was about 3% of dry mass (Fig. 2-5b). The level was probably too high for herbivores to consume, and so CLA at the top of the canopy was reduced to a lower level after June (Fig. 2-2).

Lincoln and Mooney (1984) found a significant positive correlation between the amount of herbivory and the total daily direct solar irradiance received by shrub plants, *Diplacus aurantiacus*. Their results are contrary to the results of this study, which showed a negative correlation between herbivory and light intensity. However, they found no clear relationship between the herbivory level and leaf characteristics such as the resin and nitrogen concentration, that is, possible factors regulating herbivory level. This suggests that other aspects should be taken into consideration to examine more closely the interaction between *D. aurantiacus* and its insect herbivore, *Euphydryas chalcedona*. In a field experiment, adult *E. chalcedona* oviposited more eggs on *D. aurantiacus* plants in the sun than on plants in the shade (Williams 1983). In the case of *D. aurantiacus* and *E. chalcedona*, therefore, herbivory level may be determined by oviposition preference of the adult butterfly.

Visibility for bird predation suggests an alternative hypothesis about variations in leaf herbivory level within a tree canopy. Insect herbivores at the top of a canopy are more apparent to birds than insects feeding on leaves in lower parts of the canopy. Thus it is likely that CLA at the top of a canopy would generate lower values compared to CLA in the middle or at the bottom. This may account for the spatial variations in CLA within the canopy of *F. crenata* observed after June (Fig. 2-2). However, there was no clear relationship between RPPFD (i.e. the position within the canopy) and CLA in May (Fig. 2-2). Therefore, it is difficult to explain variations in CLA observed in this research only by differences in visibility of herbivores to birds within a canopy.

Another alternative hypothesis is that high light intensities at the top of the canopy are avoided by the insects, thus causing them to go toward a

lower position in the tree. However, as mentioned above, this direct effect of light on herbivores does not explain the lack of a clear relationship between RPPFD and CLA in May (Fig. 2-2).

Finally, we must add that the insect species that consumed leaves of *F. crenata* were not identified in this study. Sixty-nine lepidopteran species were reported feeding on *F. crenata*, and the tendency for large species to feed late in the season was found within the family Noctuidae (Kamata and Igarashi 1996). There may be some difference in the species composition of insect herbivores between early in the season, immediately after leaf flush, and late in the season, after leaf expansion. Further investigation is also necessary concerning the kinds of insect species feeding on *F. crenata*.

In conclusion, spatio-temporal variation was observed in leaf herbivory level within a canopy of *F. crenata*. There were no significant differences in CLA among leaf clusters at different positions in May, immediately after leaf flush, but a negative correlation was observed between RPPFD and CLA after June. It was found that seasonal changes in the leaf characteristics, LMA and tannin concentration, affected the temporally different relationships between RPPFD and CLA.

Chapter 3: Seasonal changes in causal factors of within-tree variations in leaf herbivory level of *Fagus crenata*

3.1 Introduction

From the insect herbivore's point of view, an individual plant is a heterogeneous resource. Plants are composed of many kinds of organs, such as roots, shoots, leaves, buds, flowers and fruits, and each organ has variations in its characteristics. Insect herbivores utilize such variable plants as their food by discriminating optimal plant parts. Insect folivores also choose leaves for their use by identifying leaf characteristics. Neonate larvae face various problems after their hatch, such as host plant traits and microclimates (Zalucki *et al.* 2002), and so the location of a suitable feeding site is very important for their survival (Foster and Howard 1999). In other words, it is important for adult females to choose their optimal oviposition site. Generally, adult females prefer younger leaves to older leaves for their oviposition site, and such a preference was explained by physical characteristics of leaves (Steinbauer *et al.* 1998; Howlett *et al.* 2001; Steinbauer 2002; Nahrung and Allen 2003).

Leaves vary their characteristics according to their age, phenological traits and environments. These variations in leaf traits are observed spatially within a single plant (DeJong *et al.* 1989; Gleadow and Woodrow 2000; Rosati *et al.* 2000). Patterns of these spatial variations also change temporally. For instance, 1-year-old leaves of evergreen trees are different from current-year leaves in their characteristics, but these differences may not be so clear after a year. The variation observed

among leaves with different leaf orders in spring is not necessarily observed in summer. Moreover, although characteristics of leaves within a single tree canopy are relatively uniform in the early season immediately after leaf flush, sun leaves and shade leaves become distinct according to their light environments in the late season.

Such temporal changes in spatial differences in leaf characteristics may affect folivores differently in each period; accordingly, spatial variations in folivores level may change with time. There are many factors that cause variations in leaf traits, one of which is light intensity. While light directly influences insect herbivores in a way that they avoid direct sunlight at the top of canopy trees, it also has indirect effects on herbivores in which leaves change their traits according to their light environments. I demonstrated that within a canopy of *Fagus crenata*, light intensity affected herbivory level in the late season, one or more months after leaf flush, but that there was no clear relationship between light and herbivory level in the early season, within a month after leaf flush (Yamasaki and Kikuzawa 2003). In the early season, however, leaves were not consumed uniformly; there were variations in consumed leaf area (CLA) among leaves. There must be some other factors other than light intensity which have some effects on herbivores. I tried to clarify this point in this chapter.

I considered that leaf age and leaf order must be taken into consideration as other factors of variations in leaf characteristics. Former studies revealed effects of leaf age on insect herbivores. Although they generally prefer younger leaves (Cates 1980; Coley 1980; Raupp and Denno 1983; Coley and Barone 1996; Ikonen 2002), older leaves are preferentially eaten in some cases (Togashi and Takahashi 1977a, b).

Because *F. crenata* is a deciduous species and all leaves are of the same age, there seems no variation in leaf traits that is caused by age differences.

In terms of leaf order, leaf phenology of trees is classified into three groups, that is, flush type, successive type and intermediate type, and the leaf phenology of *F. crenata* is categorized into the group of flush type (Kikuzawa 1983). In fact, leaves of *F. crenata* flush almost simultaneously in early spring, but there are some differences in appearance timing among leaf orders and leaves on the base of shoots flush earlier than those on the chip. Flush in earlier time leads to longer exposure to herbivores, and variations in duration of exposure may have direct effects on herbivores. On the other hand, leaf order has an indirect influence on herbivores by causing variations in leaf characteristics. That is, characteristics of leaves that flush earlier may finish their changes earlier than those of leaves that flush later, and in late season, they will be less suitable for herbivores than leaves that flush later. Thus, relationships between leaf order and herbivory level were discussed on the basis of the results of my two year investigation on *F. crenata*.

Finally, I compared CLA of *F. crenata* among leaves with different leaf orders and CLAs among leaves under different light conditions separately. As for herbaceous plants, which grow leaves sequentially upward, light intensity to which leaves are exposed increases with increasing leaf order. In such a case, we can consider that the effect of light intensity on herbivores is equal to that of leaf order. In the case of woody plants such as *F. crenata*, however, leaves with different leaf orders are dispersed within a canopy, and thus the effect of light on herbivores must be examined separately from that of leaf order. Thus I discussed the effect of each factor on herbivores.

3.2 Materials and methods

This research was conducted at the Ashiu Forest Research Station, Field Science Education and Research Center, Kyoto University, located in the northeastern part of Kyoto Prefecture, Japan (35°□18′N, 135°□43′E). The mean annual temperature is 11.7□°C, and the mean annual precipitation is 2,353 mm. The forest mainly consisted of natural mixed stands of *Cryptomeria japonica* D. Don var. *radicans* Nakai, *Fagus crenata* Blume, *Quercus crispula* Blume, *Betula grossa* Sieb. et Zucc. and other deciduous species.

The research material was a Japanese beech tree, *F. crenata*, 17.2 m in height and 60 cm in diameter at breast height. A tree tower was constructed around the canopy of the material tree. Making the best use of this tower, I chose 24 leaf clusters from various positions of the canopy. A leaf cluster was defined as a group of leaves attached to shoots about one meter long. Five twigs were chosen from each leaf cluster, and I observed all leaves attached to these twigs from the time when leaves flushed till they fell, in terms of whether there were marks of herbivory or not, in 2001 and 2002.

I took digital photos of the leaves consumed by leaf chewers against the background of a white screen. Then, the area of the consumed leaves and the original leaf area were determined using the NIH Image program (developed at the U.S. National Institutes of Health), and the percentage of the consumed area was calculated from these data.

The numbers of the monitored leaves were 6,052 in year 2001, and 6,150 in year 2002. I categorized these leaves in two different ways, i.e. according to leaf order and light intensity. As shown in Fig. 3-1, I

determined four categories of leaf order: leaf order one (LO1), two (LO2), three (LO3) and four and over (LO4). Light intensity was categorized according to relative photosynthetic photon flux density (RPPFD) at the top of leaf clusters, and it was measured on an overcast day in early September, 2001 and 2002, using light meters (LI-COR, LI-190SA). Four groups under different light conditions were defined as follows, $0 < \text{RPPFD} \leq 5$ (PF1), $5 < \text{RPPFD} \leq 10$ (PF2), $10 < \text{RPPFD} \leq 50$ (PF3), $50 < \text{RPPFD} \leq 100$ (PF4).

According to the classifications described above, I categorized the observed leaves and compared CLA of leaves among groups. The data of CLA contains a lot of zero (i.e. intact leaves), and we could not assume normality. Therefore, I used nonparametric statistics, Kruskal-Wallis test, to test differences in CLA among groups, using PROC NPAR1WAY (SAS 1985). Multiple comparisons among groups of leaves were conducted using Steel-Dwass test (Kyplot, KyensLab Inc.).

Five leaves without any mark of herbivory were collected from each leaf cluster on April 17, April 30, May 22, June 26 and August 22, 2002. The leaf area of each sample was measured with a digital camera and the photo retouch software mentioned above. The samples were dried for two weeks at 40 °C, and the leaf mass per area (LMA) of each sample was calculated. The dried samples were ground with a mill, extracted with 50% methanol for 24 hours, and their total phenolics concentrations were quantified with a spectrophotometer (Shimadzu, UV-1200), using tannic acid as a standard (Price and Butler 1977; Waterman and Mole 1994). Their condensed tannin concentrations were also quantified with a spectrophotometer, using cyanidin chloride as a standard (Porter *et al.* 1986).

The measurements of LMA, total phenolics and condensed tannin were pooled by four groups of light intensity described above, and differences among four groups were tested with GLM (SAS 1985). The Scheffe's test was used for multiple comparisons among the groups.

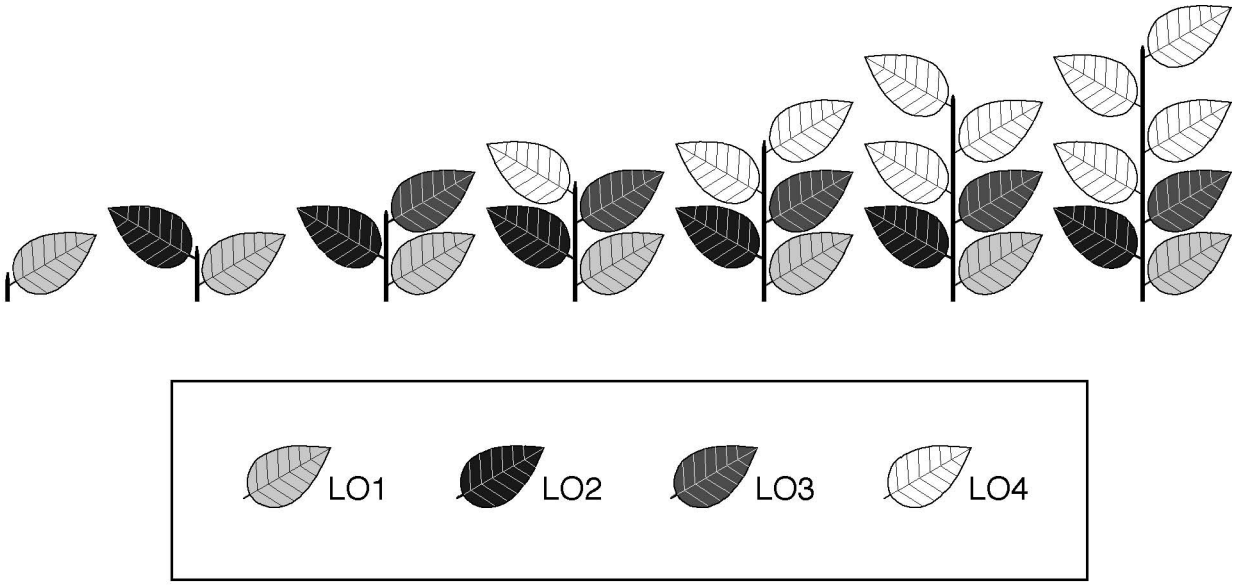

Fig. 3-1 Illustrations of current-year shoots of *Fagus crenata*. Leaves are categorized into four groups according to their leaf order. Leaves with their leaf order one, two and three are categorized into LO1, LO2 and LO3, respectively. Other leaves, whose leaf order is more than three, are categorized into LO4.

3.3 Results

Leaves of the observed *Fagus crenata* flushed at the end of April in 2001, and in mid-April in 2002. I defined the early season as a period within one month after leaf flush, and the late season as a period after one or more months after leaf flush. In year 2001, the early season corresponds to the period from the end of April to the end of May, and the late season corresponds to the period after June. In year 2002, the early and late season correspond to the period from mid-April to mid-May and the period after mid-May respectively.

Seasonal changes in frequency distribution of CLA

Frequency distributions of CLA's cumulative values in the early and late seasons of year 2001 and 2002 are shown in Fig. 3-2. In the early season of both years, the distributions of CLA skewed right, and the leaves with more than 50 % in CLA (CLAs of more than half of leaves consumed by herbivores) were very few (Fig. 3-2). In the late season of 2001, the frequency decreased with an increase in CLA in the same way as was observed in the early season, but the leaves consumed in the late season were much fewer than those in the early season (Fig. 3-2). Although the tendency of the distribution of CLA in the late season of 2002 was basically the same as that of CLA in 2001, there were some leaves that were consumed totally (CLA = 100%), and so the distribution pattern in frequency of CLA was not simple in this season (Fig. 3-2).

CLA of leaves with different leaf orders

In the early season of 2001, significant differences in CLA were found among four categories of leaf order (Kruskal-Wallis test, $P<0.0001$), and the mean values of CLA were high in LO4 compared to those of CLA in the other categories (Fig. 3-3). CLA of LO1 was significantly lower than CLAs of the other three categories in the early season (Steel-Dwass test, $P=0.0268$, $P=0.0009$, $P<0.0001$, respectively, Table 3-1). In the late season of 2001, significant differences in CLA were also found among four categories (Kruskal-Wallis test, $P=0.0193$), and the mean value of CLA was the highest in LO2 among four categories (Fig. 3-3). However, in the late season, a significant difference in CLA was detected only between LO1 and LO2 by the Steel-Dwass test for multiple comparisons ($P=0.0226$, Table 3-1).

The relationship between leaf order and CLA in 2002 was basically the same as that of year 2001. In the early season, there were significant differences in CLA among four categories of leaf order (Kruskal-Wallis test, $P<0.0001$), and CLA increased with an increase in leaf order (Fig. 3-3). I found significant differences in four pairs of comparison out of six in Steel-Dwass tests (Table 3-1). In the late season, there were no significant differences in CLA among four categories (Kruskal-Wallis test, $P=0.1186$). However, as was observed in 2001, the mean value of CLA was the highest in LO2 among four categories (Fig. 3-3).

CLA of leaves under different light conditions

While there were no significant differences in CLA among four categories of light intensity in the early season of 2001 (Kruskal-Wallis test, P=0.2711), I found significant differences among four categories in the late season (Kruskal-Wallis test, P=0.0003). The mean values of CLA decreased with an increase in light intensity (Fig. 3-4), and CLA of PF1 was significantly higher than those of PF3 and PF4 in the late season (Steel-Dwass test, P=0.0241, P=0.0003, respectively, Table 3-2).

In the early season of 2002, the differences in CLA among four categories of light intensity were significant (Kruskal-Wallis test, P<0.0001). A significant difference, however, was detected only between PF2 and PF4 by Steel-Dwass test for multiple comparisons (Table 3-2). In the late season of 2002, similar trends as those of year 2001 were observed; the mean values of CLA decreased with an increase in light intensity (Fig. 3-4). There were significant differences among four categories in the late season (Kruskal-Wallis test, P<0.0001) and CLA of PF1 was significantly higher than CLAs of the other three categories (Steel-Dwass test, P=0.0398, P<0.0001, P<0.0001, respectively, Table 3-2).

Characteristics of leaves under different light conditions

Significant differences were found in LMA among leaves under different light conditions throughout the season from April to August, 2002 (Fig. 3-5a). In April, within one month after leaf flush, the values of LMA were lower than 40 g m^{-2} in all groups of leaves under different light conditions, and the values were divided into three groups by Scheffe's test (Fig. 3-5a). In the late season, after mid-May, the differences among groups were more apparent, and an increase in LMA was observed with

increasing light intensity (Fig. 3-5a). In this season, LMA of PF4 leaves was about twice as much as that of PF1, and the values of LMA were categorized into four groups by Scheffe's test. That is, all values of four groups, PF1, PF2, PF3 and PF4, were significantly different from each other (Fig. 3-5a).

There were significant differences in total phenolics concentration of leaves among four groups under different light conditions at all sampling dates, i.e. from April to August, 2002 (Fig. 3-5b). In general, total phenolics concentrations of leaves increased with increasing light intensity. The total phenolics concentrations of leaves of PF1 were significantly lower than those of leaves of the other three categories, PF2, PF3 and PF4, except for May 22 (Fig. 3-4). Similarly, the total phenolics concentrations of leaves of PF4 were significantly higher than those of leaves of the other categories, except for April 30 and May 22 (Fig. 3-5b).

Similar trends were observed in the data of condensed tannin concentrations; they increased with increasing light intensity (Fig. 3-5c). In April 17 and April 30, the measurements of condensed tannin were divided into two groups by Scheffe's test (Fig. 3-5c). In the late season, one or more months after leaf flush, the differences among groups were more apparent. In June 26, the measurements were divided into three groups by Scheffe's test, and in May 22 and August 22, all measurements were significantly different from each other (Fig. 3-5c).

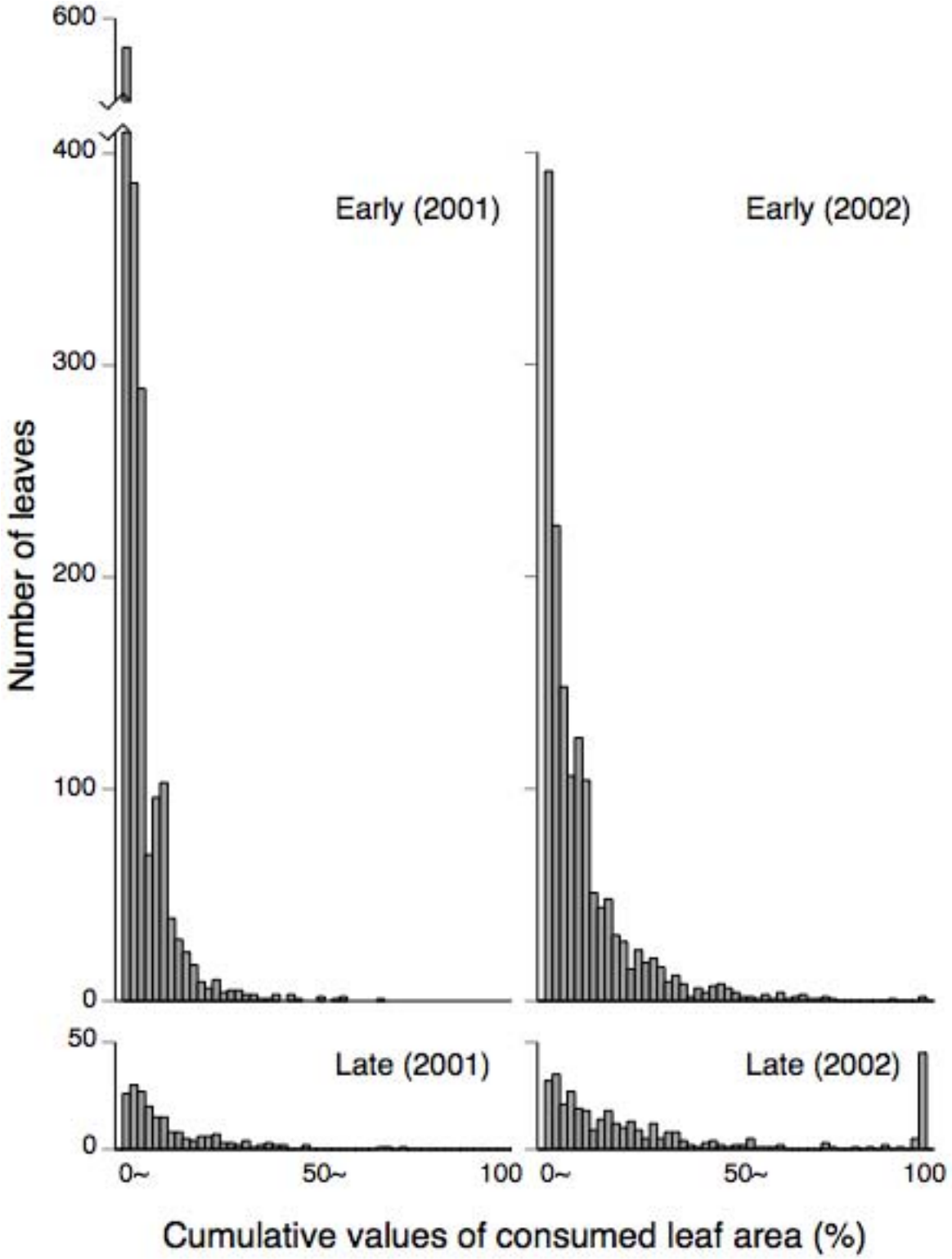

Fig. 3-2 Histgrams of consumed leaf area (CLA) in early (top) and late (bottom) seasons of year 2001 (left) and 2002 (right), with the interval size in two %. Data of the early season are shown in cumulative values of CLA within one month after leaf flush, and those of the late season are shown in cumulative values of CLA after the early season till leaves fell. Data of intact leaves (0 % in consumed area) are omitted from these graphs.

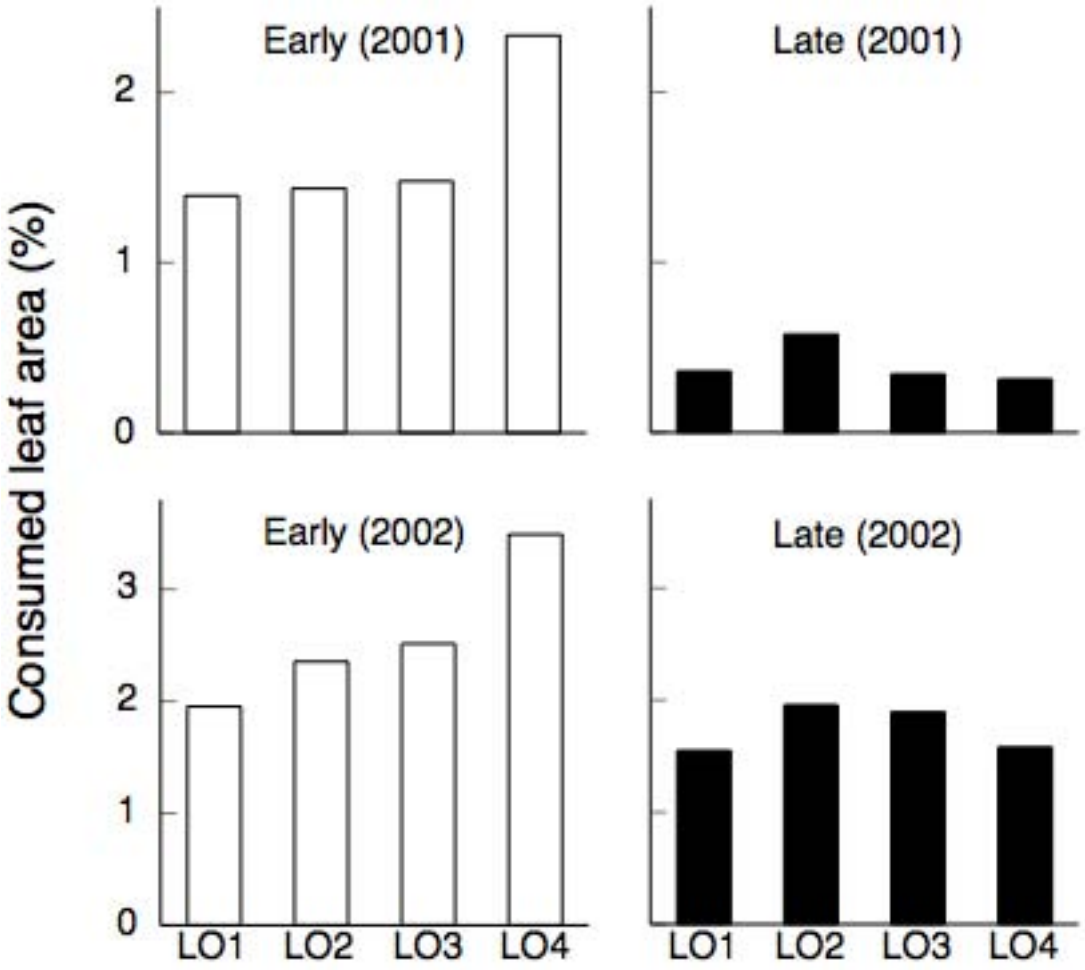

Fig. 3-3 Mean values in consumed leaf area of leaves whose leaf order is one (LO1), two (LO2), three (LO3) and more than four (LO4) in early (white bar) and late (black bar) season of year 2001 (top) and 2002 (bottom).

Table 3-1 Categories of leaf order, number of leaves of each category, and mean score in consumed leaf area of each category in the early and late season of 2001 and 2002. Means with different letters are significantly different (Kruskal-Wallis test and Steel-Dwass test, P=0.05)

	Leaf order	Year 2001			Year 2002		
		No. of leaves	Mean score in CLA		No. of leaves	Mean score in CLA	
			Early	Late		Early	Late
LO1	1	2,019	2,905 **a**	3,003 **a**	1,816	2,866 **a**	3,042 **a**
LO2	2	2,006	3,038 **b**	3,056 **b**	1,796	3,110 **b**	3,098 **a**
LO3	3	1,612	3,093 **bc**	3,020 **ab**	1,589	3,153 **bc**	3,087 **a**
LO4	≥ 4	415	3,304 **c**	3,026 **ab**	949	3,282 **c**	3,078 **a**

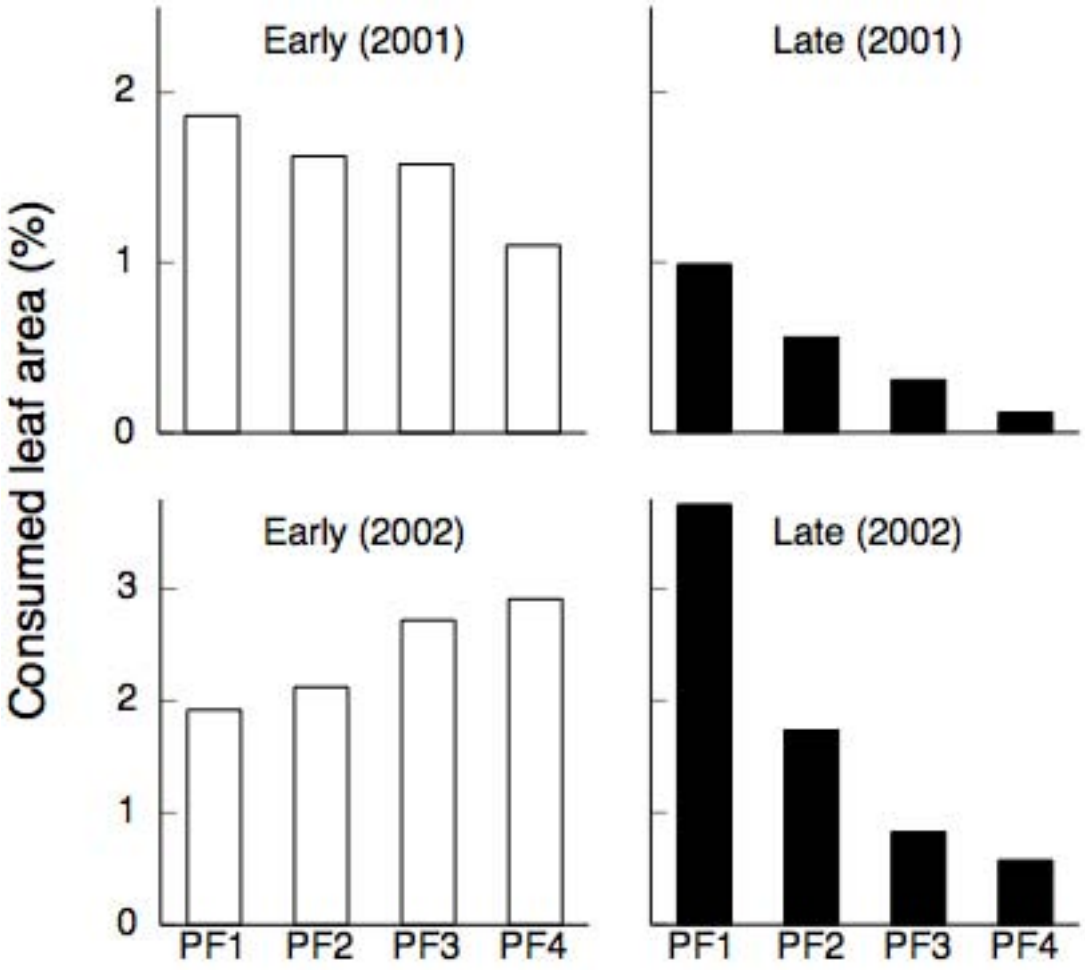

Fig. 3-4 Mean values in consumed leaf area of leaves under different light conditions, PF1 (0<RPPFD≤5), PF2 (5<RPPFD≤10), PF3 (10<RPPFD≤50) and PF4 (50<RPPFD≤100), in early (white bar) and late (black bar) season of year 2001 (top) and 2002 (bottom).

Table 3-2 Categories of light intensity, number of leaves of each category, and mean score in consumed leaf area of each category in the early and late season of 2001 and 2002. Means with different letters are significantly different (Kruskal-Wallis test and Steel-Dwass test, P=0.05)

	Light intensity	Year 2001			Year 2002		
		No. of leaves	Mean score in CLA		No. of leaves	Mean score in CLA	
			Early	Late		Early	Late
PF1	0<RPPFD≤5	1,046	2,966 **a**	3,085 **b**	1,864	2,924 **ab**	3,173 **b**
PF2	5<RPPFD≤10	1,355	3,014 **a**	3,035 **ab**	844	2,995 **a**	3,086 **a**
PF3	10<RPPFD≤50	1,832	3,070 **a**	3,020 **a**	1,603	3,153 **ab**	3,019 **a**
PF4	50<RPPFD≤100	1,819	3,027 **a**	2,993 **a**	1,839	3,199 **b**	3,021 **a**

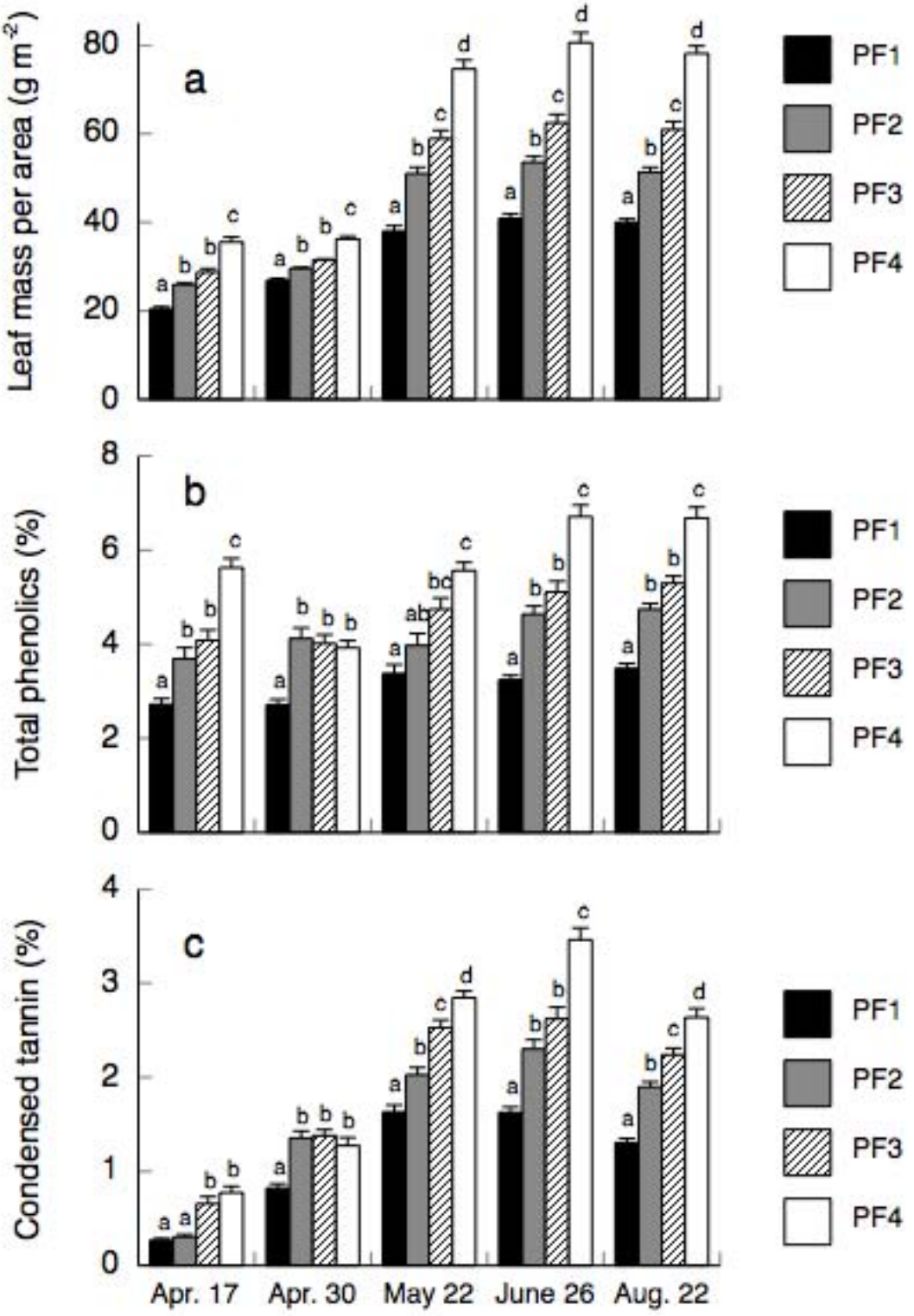

Fig. 3-5 Mean values of leaf mass per area (top), total phenolics concentration (middle) and condensed tannin (bottom) of leaves under different light conditions, 0 < RPPFD ≤ 5 (PF1), 5 < RPPFD ≤ 10 (PF2), 10 < RPPFD ≤ 50 (PF3) and 50 < RPPFD ≤ 100 (PF4) (N=35, 20, 35 and 30, respectively). Vertical bars show standard errors. Bars attached with different letters are significantly different (Sceffe's test, P=0.05).

3.4 Discussion

The continuous, non-destructive measurement of leaf areas consumed by herbivores was conducted for two consecutive years, with a canopy of *Fagus crenata* as the object. The results obtained by the measurement in each year were basically the same; there were spatial variations in CLA which changed their patterns temporally. In the early season, immediately after leaf flush, significant differences in CLA were observed among groups that were classified by leaf order (Table 3-1). CLA increased with increasing leaf order in this season (Fig. 3-3). This means that leaves that flushed later received more damage by herbivores than those that flushed earlier. However, in the late season, one or more months after leaf flush, the effect of leaf order on CLA wore off, and another factor, or the light environment of leaves, caused variations in CLA. In the late season, I found a decrease in CLA with an increase in light intensity (Fig. 3-4), which suggests that leaves in shade suffered more damage by herbivores than those under bright conditions.

Why did CLA increase with increasing leaf order in the early season (Fig. 3-3)? In other words, what accounts for the correlation between leaf order and CLA in this season? An increase in leaf order means delay in timing of leaf flush. Leaves are soft and high in water content soon after their flush (Ayres and Maclean 1987; Hunter and Lechowicz 1992; Murakami and Wada 1997), and at that time they were highly valuable for herbivores. As a result, the herbivory level generally decreases with an increase in leaf age. We can explain the results obtained in this study from this point of view. If “leaf order” is considered as a small scale pattern of “leaf age”, the results of my study are

parallel to the phenomena described above.

On the other hand, some studies indicate that leaves immediately after leaf flush are well defended with chemicals (Ossipov *et al.* 1997). It may lead to the preferential feeding of older leaves over younger leaves by insect herbivores. In the case of chrysomelid beetles, *Chrysophtharta agricola*, which feed on eucalypt, they prefer adult foliage to juvenile foliage (Lawrence *et al.* 2003; Nahrung and Allen 2003). Late instar larvae of pine moth, *Dendrolimus spectabilis*, preferentially feed on old-year needles in a season when fresh current-year needles are also available (Togashi and Takahashi 1977a, b). Moreover, leaves that flush later are exposed to herbivores over a shorter period of time than leaves that flushed earlier, and so leaves that flush later may be less damaged than those that flush earlier. This line of idea contradicts the pattern observed in this study, that is, the higher damage level in leaves that flush later.

If herbivores select leaves for consumption at the time when all leaves have already flushed, leaves that flush later may be chosen because of their high qualities for herbivores. In this case, variations in CLA of leaves depend on differences in physical and chemical characteristics of leaves. If the length of time for which leaves are exposed to herbivores is taken into consideration, leaves that flush earlier might as well be eaten heavily. The differences in the period of exposure determine the pattern of variations in CLA of leaves in this case. Leaves of *F. crenata* flush almost simultaneously in spring, and the time lag of leaf flush in a single current shoot is within a span of only a few days. However, physical and chemical characteristics of *F. crenata* leaves changed rapidly after their emergence (Fig. 3-5), and there may be great differences in characteristics among leaves that flushed one after another within a span of a few days.

In the early season, rapid changes in leaf characteristics may be more crucial than differences in exposure time in determining variations in CLA within a canopy of *F. crenata*. To clarify this point, it is necessary to measure changes in physical and chemical characteristics of leaves during the time of leaves' flush and their expansion after that in terms of their leaf order.

The replicate data of CLA in 2002 gave support to the observation in 2001, i.e. the lower values of CLA in sun leaves in the late season (Yamasaki and Kikuzawa 2003). The relationship between light intensity and CLA of leaves was clearer in 2002; CLA of PF1 (most dark categories) was significantly higher than CLAs of any other brighter category in the late season (Table 3-2). Moreover, in the early season of 2001 and 2002, the relationship between light intensity and CLA was not as clear as that in the late season (Fig. 3-4). These results are well explained by changes in the pattern of variations observed in characteristics of leaves.

In the case of LMA, there were no apparent seasonal changes during the two weeks of late-April, from April 17 to April 30 (Fig. 3-5c). However, the values of LMA increased rapidly during the following three weeks, from April 30 to May 22, and after that, we observed clear rising gradients of LMA with increasing light intensity (Fig. 3-5c). Because LMA can be considered as an indicator of leaf toughness (Choong *et al.* 1992), and toughness is considered as a defensive characteristic against herbivores (Choong 1996), it is reasonable to suppose that the pattern of variations in LMA brought out the pattern of variations in CLA (Fig. 3-4).

The spatio-temporal variations in condensed tannin concentrations of leaves also present similar patterns. In the early season, the differences among groups with different light environments were not so clear: the

measurements were categorized into only two groups by the multiple comparison test (Fig. 3-5c). In the late season, however, condensed tannin apparently increased with an increase in light intensity, and all measurements were significantly different from each other. The high values in condensed tannin mean unfavorable resources (Ayres *et al.* 1997; Nomura and Itioka 2002), which explains the spatio-temporal variations in CLA observed in this study (Fig. 3-4).

The values of total phenolics were rather constant from April to August (Fig. 3-5b). Total phenolics include condensed tannins and other chemicals such as hydrolyzable tannins. Faeth (1986) reported high hydrolyzable tannin concentrations immediately after leaf flush in evergreen oak, *Quercus emoryi*. Similarly, high concentrations in the early season and a seasonal decrease in hydrolyzable tannin were reported on mountain birch, *Betula pubescens* (Ossipov *et al.* 1997). If *F. crenata* showed a similar tendency, the rather stable pattern in total phenolics in this study is considered as the result of mixed patterns in changes of condensed and hydrolyzable tannin, i.e. a seasonal increase in condensed tannin (Fig. 3-5c) and a seasonal decrease in hydrolyzable tannin. Analysis of hydrolyzable tannin will be necessary for the general understanding of seasonal changes in phenolics of *F. crenata* leaves.

In conclusion, there were spatial variations in CLA of leaves within the canopy of *F. crenata*, and the causal factors in spatial variations changed temporally. In the early season, within one month after leaf flush, variations in CLA were observed among leaves with different leaf orders: CLA increased with increasing in leaf order. In the late season, one or more months after leaf flush, light intensity caused variations in CLA: CLA decreased with an increase in light intensity. Although physical and

chemical characteristics of leaves were different among leaf groups under different light conditions even in the early season, these differences were more apparent in the late season. We can suppose that changes in the degree of differences in leaf characteristics are main factors that cause changes in the pattern of spatial variations in CLA within the canopy of *F. crenata*.

Chapter 4: Intra-tree distribution of leaf galls, *Bunaha-magetamafushi*, within a canopy of *Fagus crenata*

4.1 Introduction

In a tree canopy, many kinds of insect herbivores use leaves as their food. Characteristics of leaves are, however, not uniform within a canopy. One obvious difference is that while sun leaves in higher position of a canopy are thick and tough, shade leaves in lower position are thin and soft (Larcher 2003). This difference becomes apparent after leaf maturation, and at that time it begins to have an effect on herbivores in the canopy. I investigated the herbivory level of *Fagus crenata*, which was 17.5 m in height, and found that in the late season, that is, in the period after leaf maturation, there were differences in herbivory level among leaf clusters under different light conditions (Yamasaki and Kikuzawa 2003). The observed differences in herbivory level were well explained by differences in leaf characteristics such as condensed tannin content.

Although Yamasaki and Kikuzawa (2003) demonstrated the correlation between spatial variations in leaf traits and the herbivory level in *F. crenata* in the late season, it did not show what affected the herbivory level in the early season, that is, in the period before leaf maturation. My aim in this chapter was to investigate what kind of interaction took place between trees and herbivores in the early season. One of the visible herbivores in the early season is gall makers. I investigated the within-tree distribution of the gall which appeared on the leaf surface of *F. crenata* immediately after leaf flush.

In Japan, 23 species are recorded for gall midges which produce galls on leaves of *F. crenata* (Tsuda 1982; Takizawa 1983). *Bunaha-magetamafushi* is one type of gall produced on the surface of *F. crenata* leaves by gall midges. It is observed on leaves early in the season; it is already produced on leaf surface even on the day of bud burst (personal observation). This suggests that adult females of gall midges select leaves in which they oviposit their eggs when leaves are still folded in buds. My research questions were how they choose buds in which they oviposit and what kind of gall distribution is observed within the canopy as a result of the oviposition preference of adult gall midges. Thus, I investigated the within-tree distribution of *Bunaha-magetamafushi*.

It is generally known that the distribution of insect galls on a tree is not uniform. Insect galls concentrate on specific trees at the stand level (Rosenthal and Koehler 1971; Eliason and Potter 2000, 2001; Kampichler and Teschner 2002), and on specific positions within a canopy at the individual tree level (Askew 1962; Rosenthal and Koehler 1971; Kampichler and Teschner 2002). Two hypotheses were proposed for this kind of unequal distribution of galls. One of them supposes that the weak flying capacity of adult gall makers limits the distribution of galls to lower positions of a canopy (Hough 1953), and the other holds that galls disperse to buds whose phenology synchronizes with adult gall makers' appearance (Askew 1962; Eliason and Potter 2000). I tried to clarify which hypothesis is applicable to the case of *Bunaha-magetamafushi*, by counting the number of galls within the canopy of *F. crenata* closely.

Previous studies on gall distributions classified leaves of canopies of their observed trees only by height (Kampichler and Teschner 2002), top, middle and bottom for example. In this study, I set up another

classification system, that is, primary shoots to which leaves were attached, according to which leaves inside the canopy were grouped. Thus, I analyzed the data of gall densities in relation both to their heights and categories of primary shoots as causal factors of gall distributions. The tall tree tower constructed around the observed tree enabled me to observe leaves at various heights of *F. crenata*.

Moreover, when we discuss the interaction between gall makers and their host plants, host plant phenology is not negligible (Ozaki 1998; Yukawa 2000). In the case of *F. crenata*, which is classified as flush type in leaf emergence pattern (Kikuzawa 1983), leaves flush within a few days. However, only one day difference in timing of leaf flush is crucial for gall midges, whose life span at the adult stage is very short, sometimes only for a day. Therefore, within-canopy variations in timing of leaf flush displayed by the day may have effects on gall distributions. I discussed the synchronization between the gall midge activity and the host tree phenology on the basis of the data of the leaf phenology obtained in several positions of the *F. crenata* canopy.

4.2 Materials and Methods

My research material was *Fagus crenata*, with 17.5 m in height and 60 cm in diameter at breast height. This study was conducted in year 2004, and the first leaf flush of the material tree in this year was observed in April 17.

Bunaha-magetamafushi is a semioval, light green color gall observed on the surface of *F. crenata* leaves (Tsuda 1982; Takizawa 1983; Yukawa and Masuda 1996). It is produced by a kind of gall midge (Diptera: Cecidomyiidae). One larva is observed inside one gall. The size of the gall is from 4.5 to 4.9 mm in diameter and 4.8 to 6.0 mm in height. The lamina of leaves with the gall on the surface winds like a wave. The gall in the canopy of the observed tree was observed on the leaf surface soon after leaf flush and dropped to the ground in late May, a month after leaf flush. I counted the number of galls in early May.

I defined primary shoots as the branches that directly ramified from the main trunk. I then traced each primary shoot from the base, and marked the point where its diameter became smaller than 5 cm. Leaves on each branch above this point were treated as one leaf cluster, and the height of each leaf cluster was determined as the average value of the top and bottom heights of each leaf cluster. Five twigs, which bore more than 20 current year shoots, were selected within each leaf cluster, and I counted the number of galls on each leaf attached to these twigs.

Although the observed *F. crenata* consisted of 17 primary shoots, only 12 shoots out of them were accessible with the tree tower, and 140 leaf clusters were found on these 12 shoots. Within these 140 clusters, I surveyed 51,413 leaves on 17,200 current year shoots. Differences in gall

densities among primary shoots and among leaves of different height classes were tested by two-way ANOVA using PROC GLM (SAS 1985).

Before the first leaf flush of the material tree, I chose three bud clusters at the bottom, middle and top of the canopy, that is, at 9.9 m, 14.2 m and 16.8 m in height respectively, and chose five twigs with more than 20 buds on each cluster. I measured the width of the buds attached to these twigs during a week before leaf flush. The measurements were done on April 9, 13 and 15 at the bottom, April 13, 15 and 17 at the middle and April 9, 13, 15 and 17 at the top of the canopy. The number of leaves was counted for these buds after their burst, and the mean value of the bud width was calculated for each category of leaf number.

From the day of the first leaf flush, the leaf flush phenology was investigated on 13 (out of 140) leaf clusters, with respect to five twigs with more than 20 current year shoots on each cluster. The proportions of the number of leaves which flushed on April 17, 18 and 19 and after April 20 to the total number of leaves were calculated respectively for each cluster.

4.3 Results

On the surface of the surveyed *Fagus crenata* leaves, I found 459 galls of *Bunaha-magetamafushi*. Figure 4-1 shows the frequency distributions of leaves with different numbers of galls (top) and current-year shoots with different numbers of gall (bottom). About 80 % of the galls (363 galls) were found solely on one leaf, and the frequencies of leaves which bore more than one gall were rather low (Fig. 4-1). More than three galls were not observed on a single leaf surface (Fig. 4-1). As for the number of galls per current-year shoot, the frequency distribution was much the same as that of the number per leaf. Among 364 current-year shoots in which galls were observed, about 80 % of the shoots (287 shoots) contained only one gall (Fig. 4-1). More than one gall in a single current-year shoot were not observed frequently, and the greatest number of galls per current-year shoot, that is, four, was observed in only two current-year shoots (Fig. 4-1).

Figure 4-2 shows the frequency distribution of current-year shoots with different leaf numbers, the mean numbers of galls per current-year shoot with different leaf numbers and the mean numbers of galls per leaf with different leaf orders. The leaf numbers per shoot ranged from one to eight on the investigated *F. crenata*, and three leaves per current-year shoot was the most frequently observed (Fig. 4-2a). A bimodal distribution was observed in the mean numbers of galls per current-year shoot with the two peaks in NL3 and NL5, and no gall was observed in NL7 and NL8 shoots (Fig. 4-2b). In general, the mean numbers of galls per leaf with different leaf orders distributed normally, with the peak found in leaves in the central position: in the case of NL3 shoots, the peak of the number of galls was

observed in LO2 leaves, and in NL4 and NL5 shoots, galls were the most abundant in LO3 leaves (Fig. 4-2c).

Figure 4-3 shows the frequency distributions of the leaf clusters attached to 12 primary shoots of *F. crenata* at different heights. The leaf clusters were observed within 5 m and 18 m in height (Fig. 4-3). Shoots F, G and H were epicormic shoots with only one or two leaf clusters (Fig. 4-3). Shoots A, D, E and J were rather small shoots with less than 10 leaf clusters (Fig. 4-3). Shoots B, C and I were big shoots with more than 15 leaf clusters (Fig. 4-3). The main trunk of the observed *F. crenata* branched at 9.57 m in height into two shoots, that is, shoots K and L (Fig. 4-3).

The mean numbers of galls per shoot calculated for 10 categories of primary shoots are shown in Fig. 4-4. The data of shoots F, G and H were pooled in this figure. The number of galls per shoot was the biggest in epicormic shoots, shoots F, G and H (Fig. 4-4). Intermediate values were observed in the number of galls per shoot of rather small shoots (shoots A, D, E and J) and big shoots in lower position at their base (shoots B and C) (Fig. 4-4). The shoots in higher position at their base (shoots I, K and L) were low in the number of galls per shoot, and especially in shoots K and L, galls were very few (Fig. 4-4).

The relationship between the height of leaf clusters and the number of galls per shoot is shown in Fig. 4-5. On the whole, the values of gall number are dispersed, and the range of the dispersion changed in accordance with the change in height. In lower positions of the canopy, below nine meter in height, the range of the dispersion of gall numbers broadened with increasing height, and in higher positions of the canopy, above nine meter in height, it narrowed with increasing height (Fig. 4-5).

At the top of the canopy, above 14 meter in height, the numbers of galls were very low (Fig. 4-5).

The results of two-way ANOVA using the data of the leaf clusters attached to shoots with more than 10 leaf clusters are shown in Table 4-1. Significant differences were found in the number of galls per shoot both among leaf clusters attached to different primary shoots and among clusters at different heights, although there was no interaction between these two factors (Table 4-1). The number of galls was significantly higher in shoot C than those in shoots I, K and L (Table 4-1). In regard to the height of leaf clusters, the numbers of galls in leaf clusters in lower positions, below 10 m in height, were significantly higher than those in clusters in higher positions, above 14 m in height (Table 4-1).

The proportions of buds with different numbers of leaves in different vertical positions are shown in Fig. 4-6. The proportions of buds with less than three leaves (NL1-2) decreased with increasing height, and the proportions of buds with more than three leaves (NL4-8) increased with increasing height (Fig. 4-6). The proportions of buds with three leaves (NL3) were about 45 %, irrespective of the height (Fig. 4-6).

Figure 4-7 shows temporal changes in the frequency distribution of bud width before bud burst in different layers of the canopy, top, middle and bottom, with categorization of the buds by leaf number into three groups, i.e. buds with less than three leaves (NL1-2), buds with three leaves (NL3) and buds with more than three leaves (NL4-6). Although normal distributions were observed in the bud width in all layers, there were differences in the peak value of bud width among the layers on each measurement day, and there were also changes in the peak value with time until two days before bud burst (Fig. 4-7). As is shown in Fig. 4-6, there

were differences in the proportion of buds with different leaf numbers among the layers; the percentages of NL1-2 buds were higher at the bottom than those in the upper two layers, and the percentages of NL4-6 buds were higher in the upper two layers than those at the bottom (Fig. 4-7). When we compared the size of bud width among buds in the three categories of leaf number in different layers, there were also differences in the peak value and size distribution among the layers. This was true even when we took account of the one-day difference in the timing of bud burst. For example, the width of NL3 buds distributed from 2.0 to 3.4 cm at the bottom, from 2.6 to 4.2 cm at the middle and from 2.8 to 4.2 cm at the top of the canopy respectively at the dates two days before bud burst (Fig. 4-7); that is, the bud width of the upper two layers was bigger than that of the bottom layer when we compared the values at these dates.

The leaf phenology of 13 leaf clusters which belong to shoots C and L is shown in Fig. 4-8. In the lower part of shoot C, below 11.5 m in height, more than half of the leaves flushed in April 17 (Fig. 4-8). More than half of the leaves flushed by April 18 in the clusters of shoot C whose height is below 14 m, and in the higher part of shoot C, above 14 m in height, the great majority of the leaves flushed in April 18 and 19 (Fig. 4-8). In shoot L, the percentages of the leaves which flushed by April 18 decreased with increasing height (Fig. 4-8).

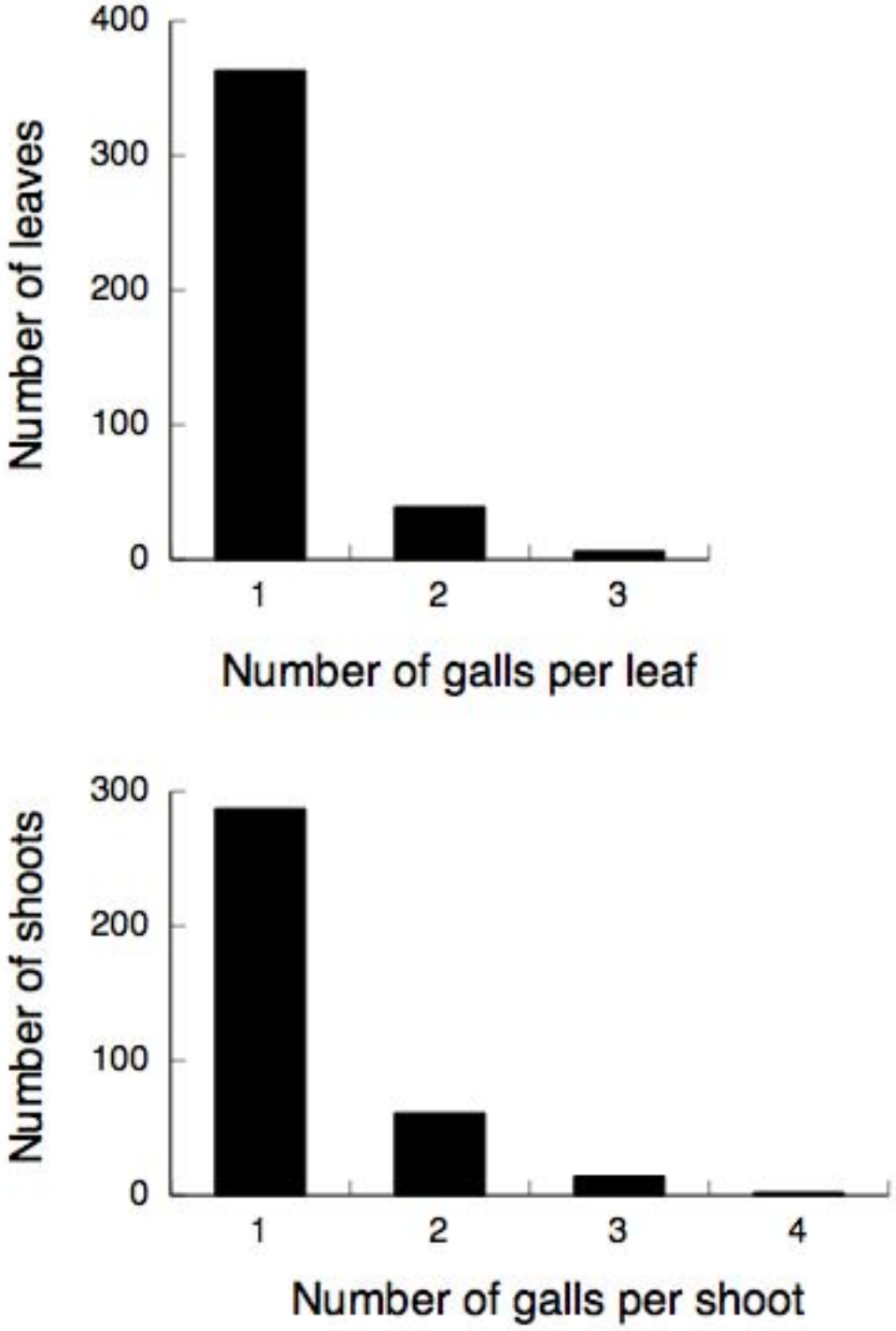

Fig. 4-1 Frequency distributions of leaves with different numbers of galls (top) and current-year shoots with different numbers of galls (bottom).

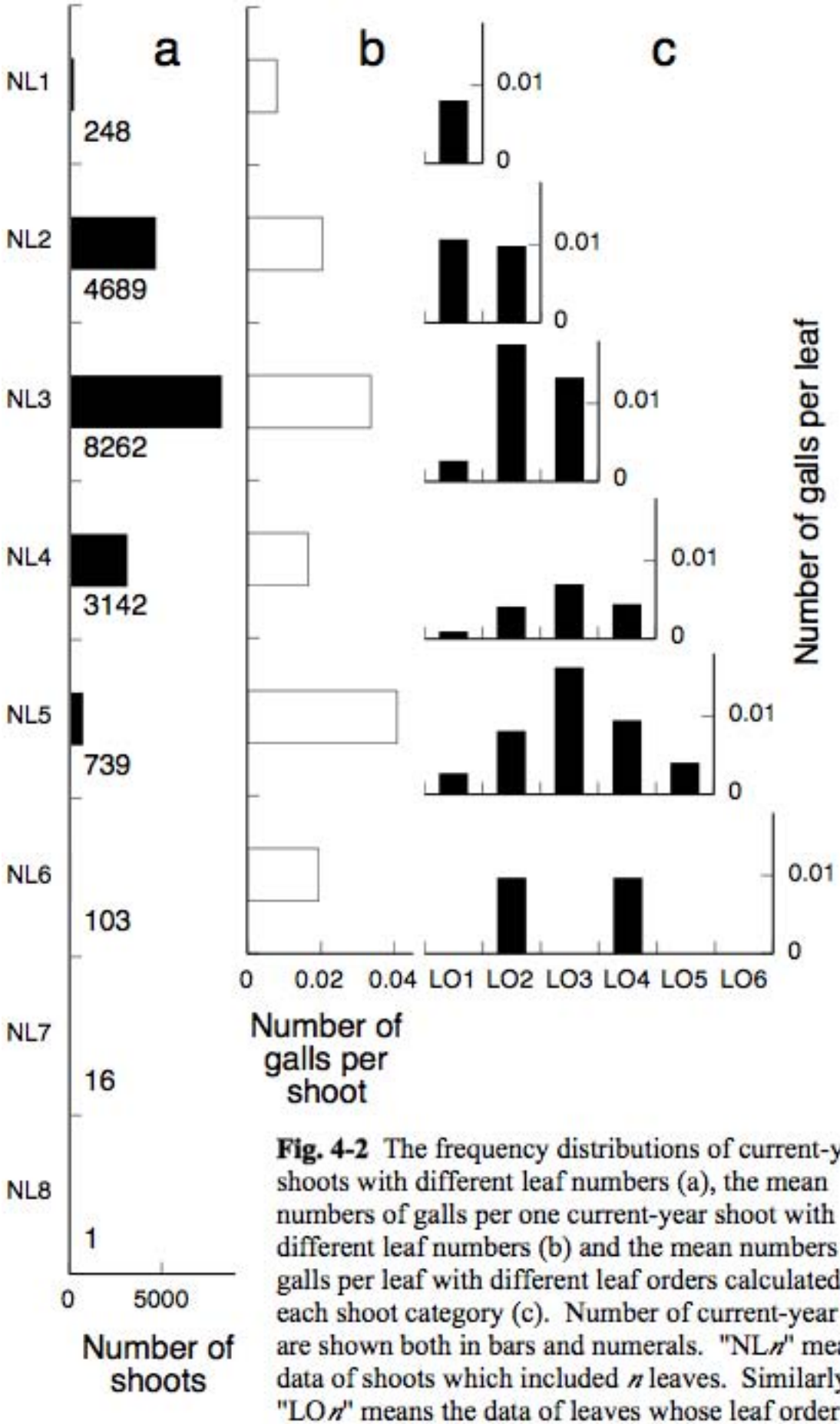

Fig. 4-2 The frequency distributions of current-year shoots with different leaf numbers (a), the mean numbers of galls per one current-year shoot with different leaf numbers (b) and the mean numbers of galls per leaf with different leaf orders calculated for each shoot category (c). Number of current-year shoots are shown both in bars and numerals. "NL*n*" means the data of shoots which included *n* leaves. Similarly, "LO*n*" means the data of leaves whose leaf order is *n*.

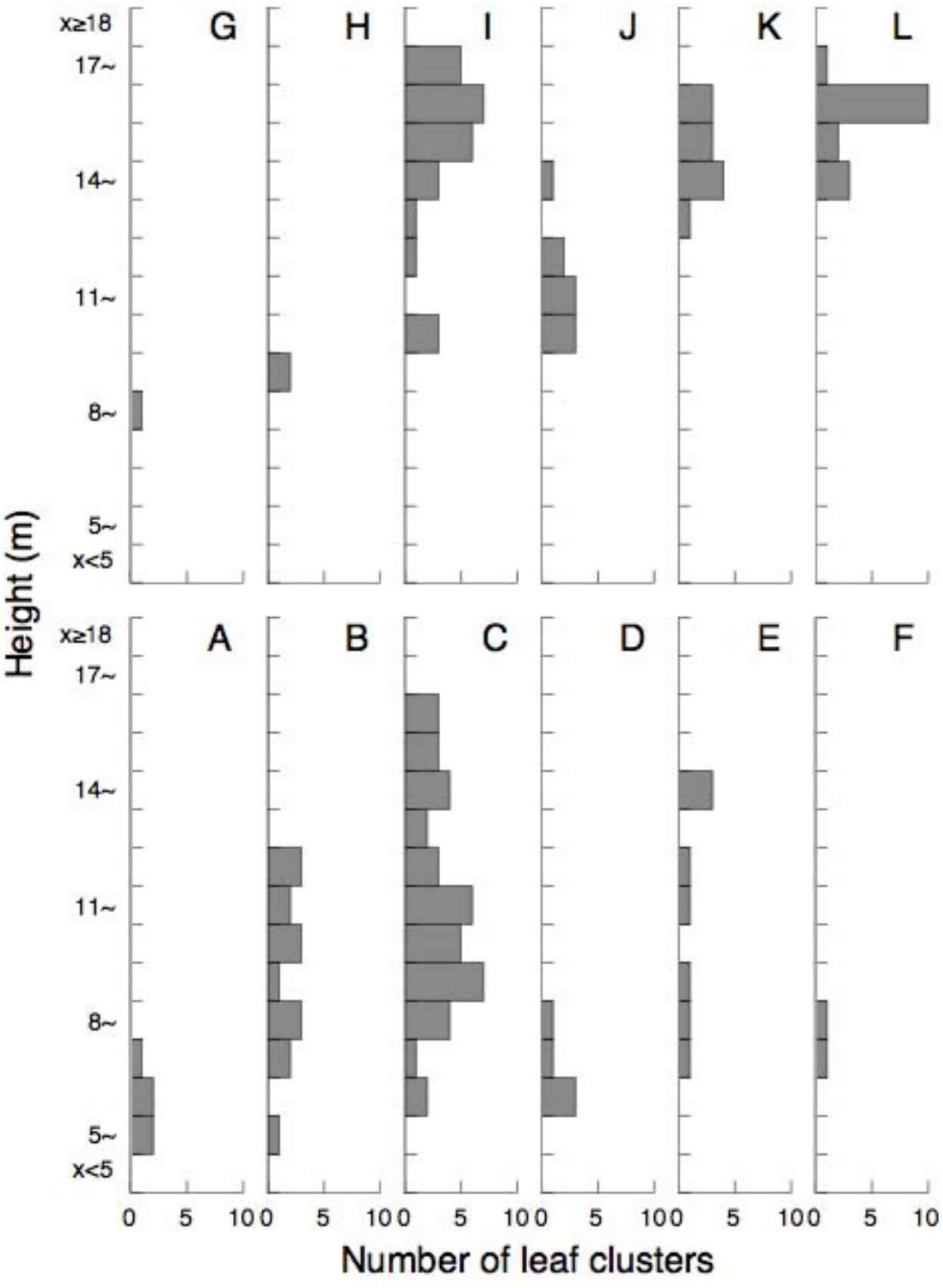

Fig. 4-3 Frequency distributions of leaf clusters at different heights attached to 12 primary shoots (from A to L) with the interval size in one meter.

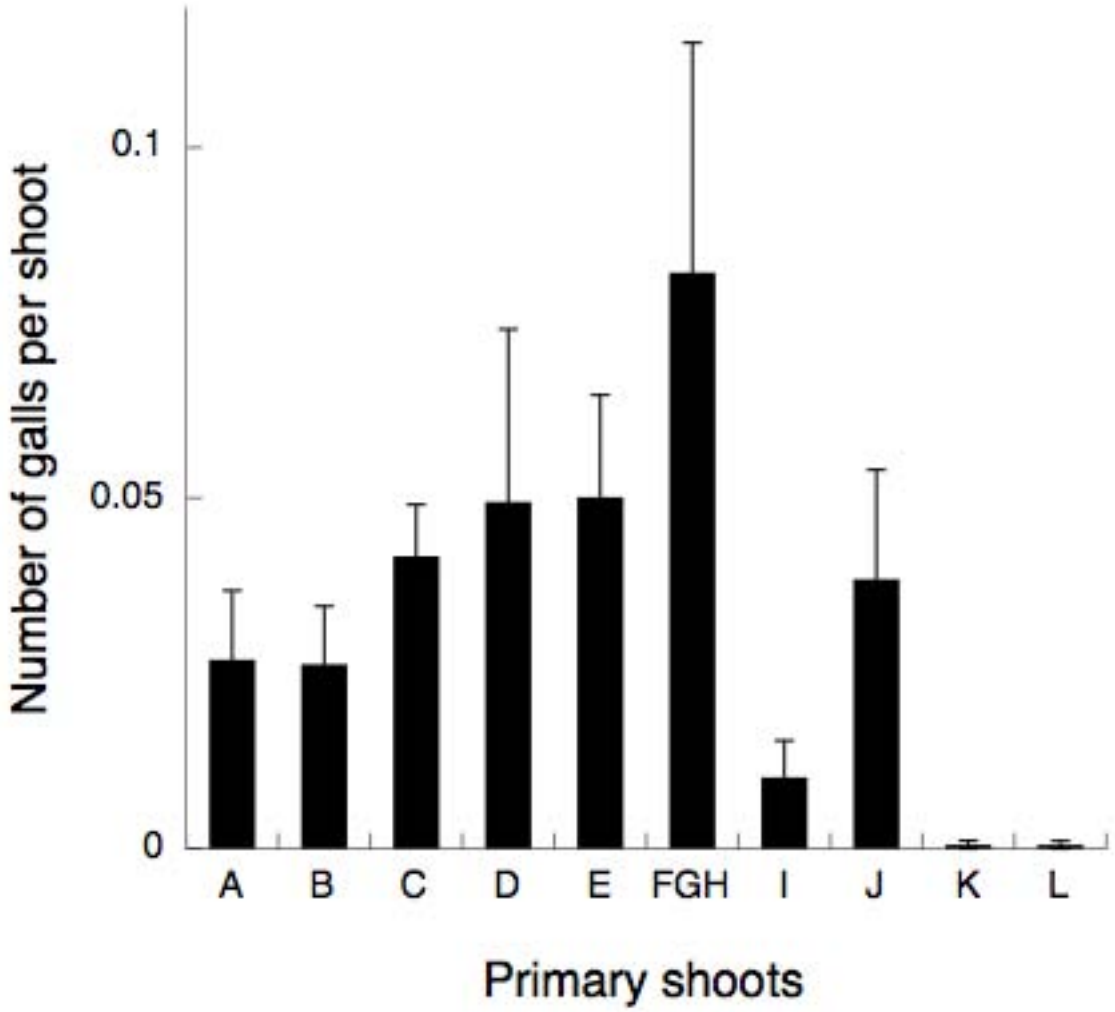

Fig. 4-4 Mean numbers of galls per current-year shoot of *Fagus crenata*. Means are calculated for leaf clusters categorized by 12 primary shoots. Vertical bars show standard errors (N=5 for A, 15 for B, 40 for C, 5 for D, 8 for E, 5 for FGH, 26 for I, 9 for J, 11 for K and 16 for L, respectively).

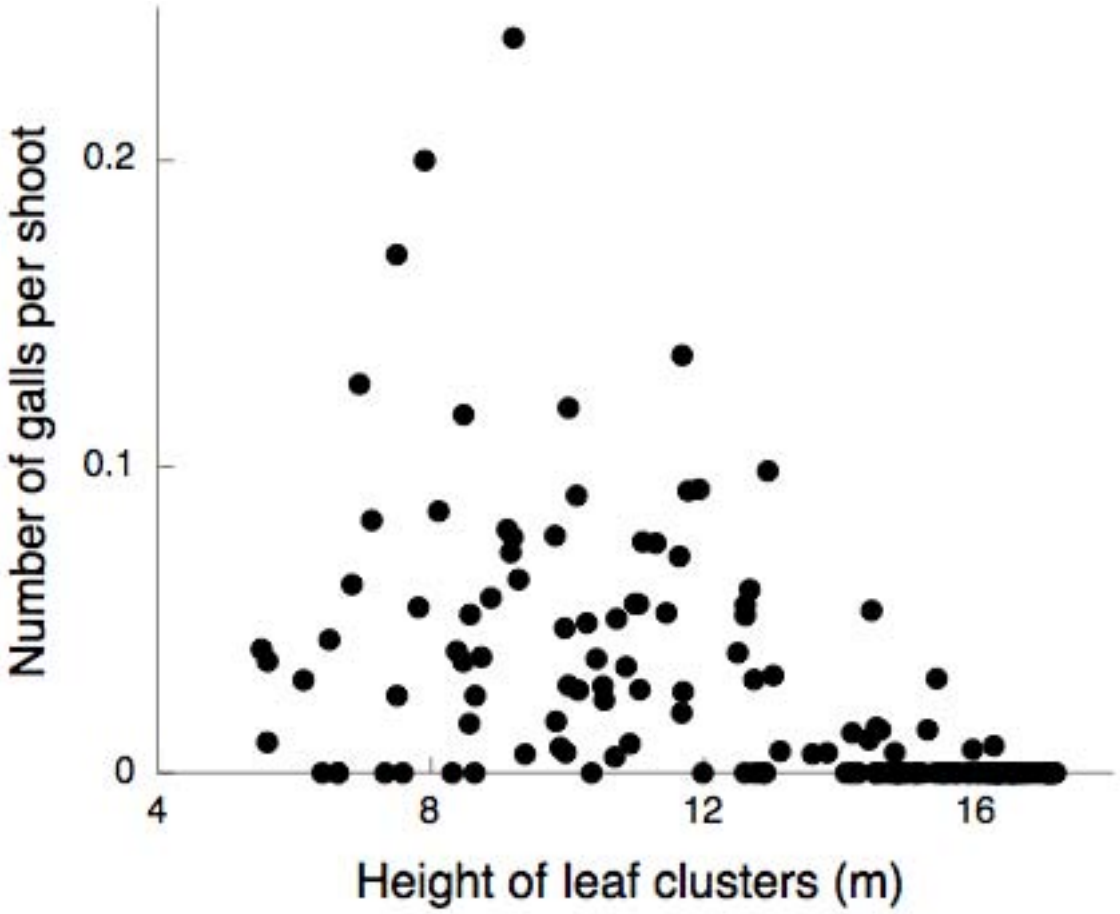

Fig. 4-5 The relationship between the height of leaf clusters and the number of galls per current-year shoot in the canopy of *Fagus crenata*.

Table 4-1 Results of two-way ANOVA on the number of galls per shoot with branches and heights as factors. The numbers of galls are shown in means ± standard errors. The means followed by different alphabetical letters are significantly different (Scheffe's test, P<0.05).

Source	d.f.	F	P	n	Number of galls per shoot
Branch	4	8.92	<0.0001		
B				15	0.0263 ± 0.0083 **ab**
C				40	0.0417 ± 0.0074 **a**
I				26	0.0102 ± 0.0052 **b**
K				11	0.0006 ± 0.0006 **b**
L				16	0.0006 ± 0.0006 **b**
Height	5	6.48	<0.0001		
~ 8 m				6	0.0560 ± 0.0241 **a**
8~10 m				15	0.0488 ± 0.0154 **a**
10~12 m				19	0.0460 ± 0.0070 **ab**
12~14 m				11	0.0277 ± 0.0098 **ab**
14~16 m				28	0.0031 ± 0.0014 **b**
16 m ~				29	0.0003 ± 0.0003 **b**
Branch * Height	9	1.07	0.3924		

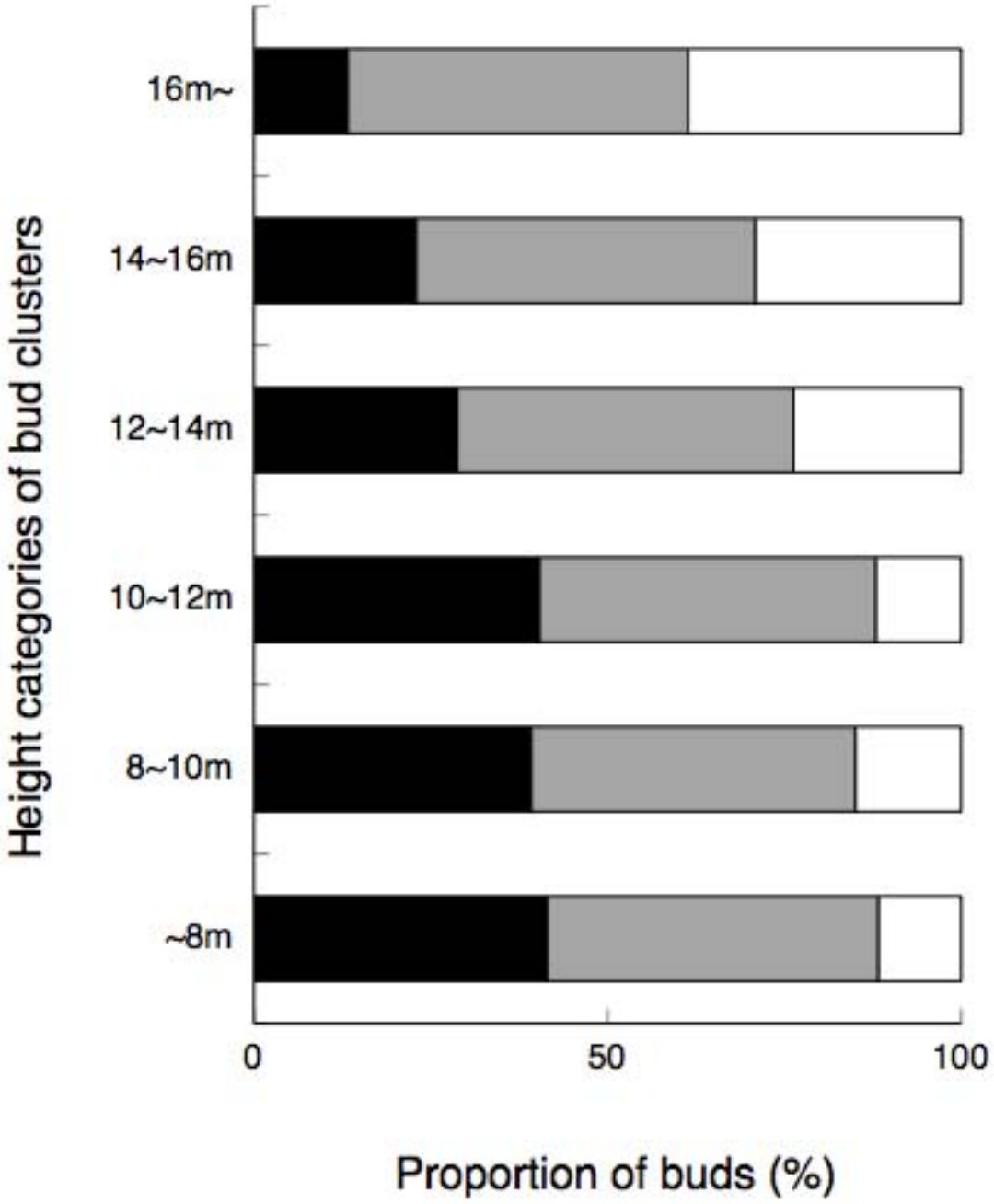

Fig. 4-6 Proportions of buds with different numbers of leaves at different heights. Mean values were calculated for different groups of bud clusters categorized by height. Black, gray and white bars show the data of buds with less than three leaves (NL1-2), the data of buds with three leaves (NL3) and the data of buds with more than three leaves (NL4-8), respectively.

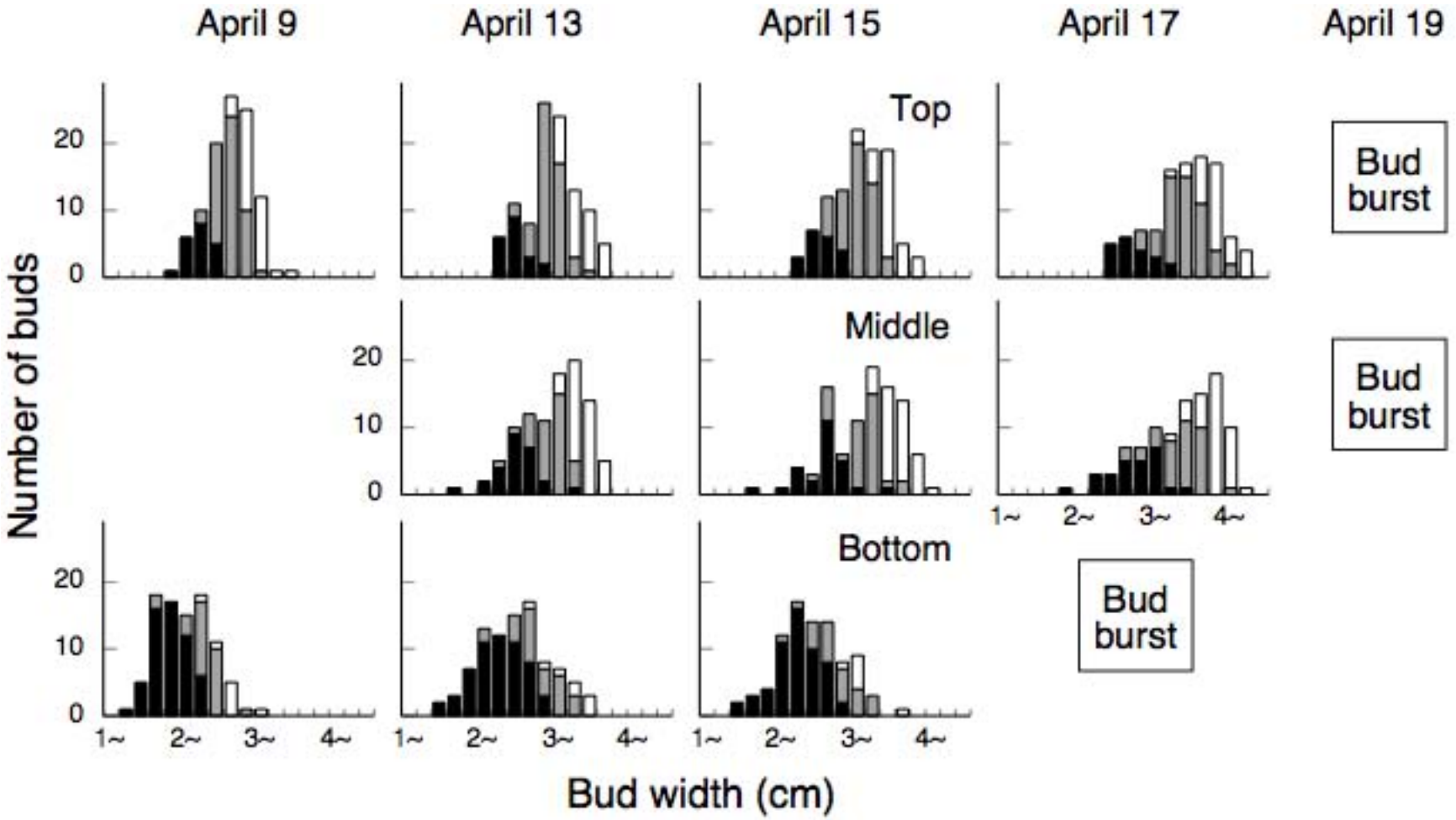

Fig. 4-7 Changes in frequency distribution of bud width before bud burst with interval in 0.2 cm. The data of bud clusters from the top (top, N=103), middle (middle, N=98) and bottom of the canopy (bottom, N=92) are shown. The dates of measurements are shown at the top, and the dates of bud burst are indicated by square signs for each canopy layer. Black, gray and white bars show the data of buds with less than three leaves (NL1-2), the data of buds with three leaves (NL3) and the data of buds with more than three leaves (NL4-6) respectively.

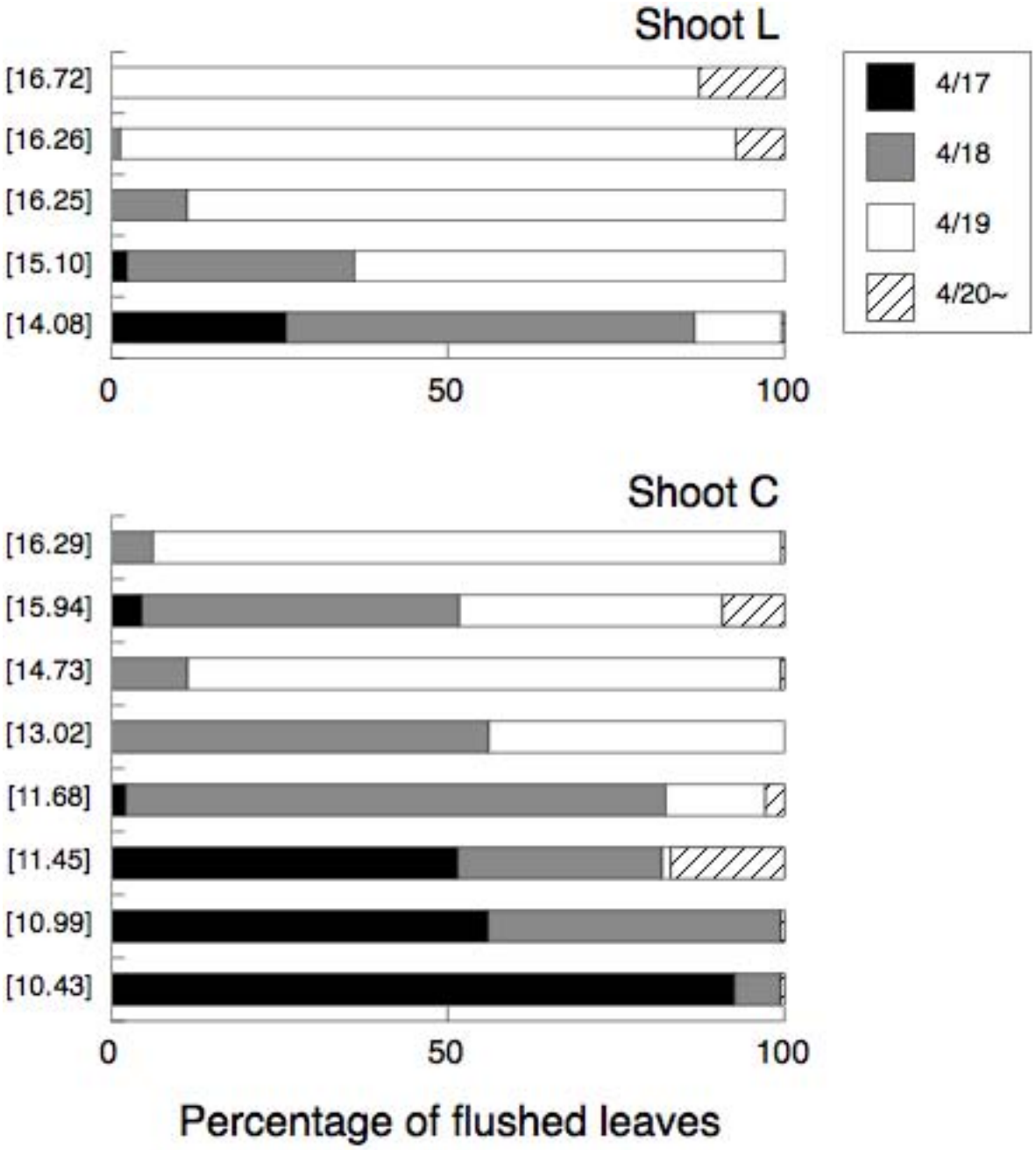

Fig. 4-8 Percentages of *Fagus crenata* leaves which flushed in April 17 (black bar), April 18 (gray bar), April 19 (white bar) and after April 20 (white bar with slant lines), 2004. The data of five leaf clusters which belong to the upper branch L (top) and the data of eight leaf clusters which belong to the lower branch C (bottom) are shown. The numerals in parenthesis on the left side of each bar graph show the height of each leaf cluster in meter.

4.4 Discussion

I surveyed the within-tree distribution of galls, *Bunaha-magetamafushi*, in a single tree canopy of *Fagus crenata* as a material. Variations in the number of galls were observed among current-year shoots at different heights and among current-year-shoots attached to different primary shoots. Nearly 80 % of the galls were produced solely on a single leaf of *F. crenata* (Fig. 4-1). Moreover, nearly 80 % of the buds with galled leaves contained only one gall (Fig. 4-1). Taking these phenomena and the early appearance of the gall (immediately after leaf flush) together, it can be assumed that adult females of the gall midges oviposited their eggs once in one bud before bud burst. The following discussion will be based on the assumption that the gall midges demonstrated their oviposition preference at the bud stage of *F. crenata*.

Because most of the adult gall midges oviposited their eggs once in one bud (Fig. 4-1), it seems that there was no competition for resources among galls. Therefore, it seems unnecessary for the gall midges to choose bigger buds for their oviposition sites. The results obtained in this study supported this line of idea. Generally, buds with more leaves were bigger in their width (Fig. 4-7). If the gall midges selected buds as their oviposition sites by bud size and they preferred bigger buds, the numbers of galls per bud will increase with an increase in leaf number. As shown in Fig. 4-2b, such a trend was not observed. Although the number of galls per shoot was the biggest in the category of buds with five leaves, an increase in gall density with more leaves within buds was not observed. When we categorized the observed shoots by the number of leaves per

shoot, there was a significant positive correlation between the number of shoots and the number of galls ($r=0.965$, $p<0.01$). It means that adult gall midges did not select buds in which they oviposit by bud size, and they oviposited eggs equally on buds of various sizes.

Then, what are the factors that caused the unequal within-tree distribution of galls? There were differences in gall densities both among leaf clusters attached to different primary shoots (Fig. 4-4) and among leaf clusters that belong to different height classes (Fig. 4-5). Gall densities were very low on two primary shoots, shoots K and L (Fig. 4-4), which were located at the top of all primary shoots (Fig. 4-3). The numbers of galls were very small at the top of the canopy, above 14 m in height, and most leaf clusters contained no gall in this higher position (Fig. 4-5). The results of two-way ANOVA indicated that both the primary shoots and the heights of leaf clusters had significant effects on the number of galls per shoot, though no interaction between the two factors was observed (Table 4-1). This indicates that (1) gall densities were significantly different among leaf clusters attached to different primary shoots, (2) gall densities were significantly different among leaf clusters at different heights, and (3) the number of galls changed according to the change in height (in this case, galls were fewer with increasing height) irrespective of which primary shoots leaf clusters were attached to.

Moreover, multiple comparison tests show that (1) primary shoots in higher positions were low in gall densities than those in lower positions and (2) gall densities were lower with increasing height. It seems natural that primary shoots in higher positions are low in gall densities because the shoots consisted of leaf clusters in higher positions whose gall densities were low. However, significant effects of both primary shoots and

heights were found by two-way ANOVA. This suggests that gall densities of leaf clusters were different even among leaf clusters at the same height if the clusters belonged to different primary shoots.

There are two hypotheses proposed for the unequal distribution of insect galls within plants. One supposes that the low ability in flight of adult gall midges limits the distribution of galls to lower positions (Hough 1953). In fact, an increase in gall densities was found with decreasing height of leaf clusters in the canopy of *F. crenata* (Table 4-1). However, as was discussed above, we cannot explain the results obtained in this research only by the factor of height, that is, "weak flying capacities" of gall midge adults.

The other hypothesis for the unequal distribution of insect galls within plants predicts that a close synchrony between bud burst and adult emergence is important for many gall-inducing insects, particularly for those that oviposit before leaf flush (Askew 1962; Eliason and Potter 2000). That is to say, the timing of adult eclosion synchronizes with the timing of bud burst, and galls concentrate in specific positions where conditions of leaves are optimal for oviposition. Because *F. crenata* is classified as flush type in leaf emergence pattern (Kikuzawa 1983), there seems no variation in leaf flush timing within a single tree canopy. However, I found variations in the timing of leaf flush among leaf clusters attached to different primary shoots and within a single primary shoot. There were also variations in the timing of leaf flush among leaf clusters at different heights. In general, leaves which belonged to primary shoots in lower positions flushed earlier than those in higher positions, and a similar tendency was observed within a single primary shoot (Fig. 4-8). This pattern corresponds to the differences in gall densities among primary

shoots and among leaf clusters at different heights (Table 4-1). Thus, it can be supposed that the adult gall midges oviposited their eggs on buds which burst earlier within the canopy of *F.crenata*.

Gall midge species that produce *Bunaha-magetamafushi* have not yet been identified, and their life history is not clear. It seems that there is an optimal location within buds for oviposition. The high gall densities on leaves in the central position within buds (Fig. 4-2c) may indicate some characteristic of adult gall midges' oviposition behavior. They may exhibit their oviposition preference for a location within buds in which galls may develop successfully. However, further studies of the life history of the gall maker of *Bunaha-magetamafushi* are necessary for more detailed discussion about this topic and the results obtained in this study.

In summary, unequal within-tree distributions of *Bunaha-magetamafushi* were observed inside the canopy of *F. crenata*. Gall densities were higher on primary shoots in lower positions than those in higher positions, and within a single primary shoot, gall densities were higher in lower leaf clusters than those in higher leaf clusters. These variations in gall densities corresponded to variations in leaf phenology. This suggests that the appearance and oviposition behavior of adult gall midges synchronized with the earlier flush of buds in the canopy.

Chapter 5: General discussion: Seasonal changes in the mode of effects on herbivores

I investigated within-tree distributions of herbivory level, *Fagus crenata* as my research material. In general, herbivores used leaves unequally within the canopy of *F. crenata*, and the selection of leaves by herbivores was carried out in the way in which they maximize their performance. The position of leaves that herbivores preferred depended on when, in a year, they made use of leaves. That is, there were seasonal changes in the mode of effects of plant properties on herbivores.

The selection of leaves for use by herbivores started in the very early season, before the flush of leaves. In Chapter 4, I investigated within-tree distributions of the gall, *Bunaha-magetamafushi*, produced by gall midges, and found significant effects of the primary shoots and heights of leaves on gall densities. The results were well explained by a close synchronization between the appearance of gall midges and the leaf phenology of *F. crenata*. Although leaves of *F. crenata* flushed almost simultaneously in the canopy, leaves in different positions varied in the timing of bud burst. That is, leaves attached to primary shoots in lower positions within the canopy flushed earlier, and leaves in lower positions within a single primary shoot flushed earlier. Gall densities were high in leaf clusters with those leaves which flushed earlier in the canopy.

Immediately after the leaf flush of *F. crenata*, variations in herbivory level were observed among leaves that flushed at different times. Chapter 3 investigated changes in leaf area consumed by herbivores within the canopy of *F. crenata*. It revealed the effect of leaf order on herbivores;

there was an increase in consumed leaf area with increasing leaf order within a month after leaf flush. It was thus supposed that leaf order indirectly affected herbivores through changing characteristics of leaves with different leaf orders.

One or more months after leaf flush, there were changes in factors that caused within-tree variations in herbivory level; light intensity had significant effects on herbivory level in this season. In Chapters 2 and 3, I clarified the effect of light intensity on leaf herbivory level with my continuous, non-destructive observation of over 6,000 leaves within the canopy of *F. crenata*. A decrease in consumed leaf area with increasing light intensity was observed one or more months after leaf flush. In this season, differences in leaf characteristics such as LMA and condensed tannin concentration supposedly caused within-tree variations in consumed leaf area.

As shown in Fig. 1-1, there are three kinds of factors that cause within-tree variations in leaf herbivory level, namely, plant internal characters, plant external characters and plant environmental properties. This research demonstrated that these factors had different effects on herbivores in the canopy of *F. crenata* at different times of year. In the canopy of *F. crenata*, plant external characters and environmental properties had direct effects on herbivores early in the growing season. That is, densities of insect galls on leaves were affected by the primary shoots to which leaves were attached and heights of leaves. After the flush of leaves, indirect effects of plant external characters on herbivores were observed. In this season, the difference in the time of leaf flush among leaves with different leaf orders seem to have influenced leaf

herbivory level through the mediation of differences in leaf characteristics. In the late season, after leaf maturation, plant environmental properties had indirect effects on leaf herbivory level. That is to say, leaf herbivory level was affected by variations in leaf physical and chemical characteristics caused by differences in light intensity within the canopy.

In sum, plant environmental properties and plant external characters changed the ways of affecting herbivores with time. Plant external characters had direct effects on herbivores in the very early season, before leaf flush, and had indirect effects on herbivores in the early season, after leaf flush, in the canopy of *F. crenata*. In a similar way, plant environmental properties had direct effects on herbivores in the very early season, and had indirect effects on herbivores in the late season, after leaf maturation. Thus there were transitions in the mode of effects on herbivores from direct to indirect one.

I will now consider why such transitions from direct to indirect effects were observed. In the very early season, before leaf flush, leaves were folded inside buds and it must have been difficult for herbivores to detect internal differences. Therefore, it seems natural that herbivores were only directly affected by plant environmental and external factors in this season. Immediately after leaf flush, variations in leaf internal characters caused by external characters, that is, leaf orders, influenced herbivores. As shown in Fig. 3-4, the differences in leaf internal characters among leaves in different positions in the early season were not as big as those in the late season. Therefore, the effect of plant internal characters on herbivory level was not so remarkable in the early season. As time passed, differences in leaves' internal characters arose from their different environments and their effects on herbivory level became more

apparent. Thus in the late season, after leaf maturation, plant environmental properties started to have an indirect influence on herbivores.

Since the leaf emergence pattern of *F. crenata* is flush type, leaves flush almost simultaneously in spring. Is this characteristic desirable or undesirable for trees in terms of defense mechanism from herbivores? In the case of trees of flush type in leaf emergence pattern, leaves at the top of canopies are, once they have maturated, well defended from herbivores because of their inconvenient properties for herbivores. Although canopy expansion occurs for only a limited time in spring, well defended sun leaves whose photosynthetic rates are high enable the canopy to acquire high photosynthetic gain in the late season, after leaf maturation. In the case of trees of successive type in leaf emergence pattern, the top of canopies is covered with new leaves over a longer period of time. This means that they suffer more damage by herbivores because new leaves contain properties favored by herbivores. On the other hand, trees of this type have the advantage of a high rate of canopy expansion with their successive leaf emergence. The above description of the two types of tree suggests that there is a trade-off between canopy expansion and defense mechanism. Further investigations on spatio-temporal variations in leaf herbivory level observed in trees of different types in leaf emergence pattern will reveal how this trade-off occurs.

I categorized the effects of plant properties on leaf herbivory level into five types (Fig. 1-1) as follows:

type A: direct effects of plant environmental properties
type B: direct effects of plant morphological characters
type C: indirect effects of plant environmental properties through changes in plant morphological characters
type D: indirect effects of plant morphological characters through changes in plant internal characters
type E: indirect effects of plant environmental properties through changes in plant internal characters

In the case of the investigated *F. crenata*, effects of type A and B were observed on the gall densities within the canopy in the very early season. In the early season, within one month after leaf flush, effects of type D were observed on the leaf herbivory level. In the late season, one or more months after leaf flush, effects of type E were observed on the leaf hrbivory level. Effects of type C were not observed in the investigated canopy of *F. crenata*. Among possible factors that have this kind of effect on herbivory level is the difference in proportion of buds with different leaf numbers at different heights (Fig. 4-6). There may be some herbivores that tend to be affected in the way of type C. Moreover, investigations on specific herbivores of *F. crenata* will find other elements that are involved in the effects described above.

Finally, a number of species have been reported for leaf gall makers and folivores which live inside a canopy of *F. crenata*. Although they differ in their growing season and the way of resource utilization, they make use of leaves of their canopies equally successfully. It can be supposed that each species has its own way of adapting to its host tree's

spatio-temporal variations in various factors. Further studies on specific herbivore species of *F. crenata* will show how different species are affected by their host trees' spatio-temporal variations.

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